Predicting safe sandwich production

P. 017

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Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

[P.001]

Joint application of antimicrobial agents on microbial flora chilled meat cattle. Use of mathematical models

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Application of mathematical models to quantify and predict microbial growth in meat is useful tool because the control thereof is critical.

In order to extend the useful life, various technologies have been implemented that acting jointly have a greater action, than if used separately, as is the case of UVC light and essential oils.

The objectives of this work were: i) to study the effect of the combined application of antimicrobial agents on samples of beef with polyethylene low density films and stored at refrigeration temperatures, on development of spoilage microorganisms; ii) to model mathematically this microbial growth and iii) validate the models with own experimental results.

Samples of beef *Longissimus dorsi* muscle pH 5.8 were used. Samples were irradiated with UVC light for 5 minutes and then were added 1 ml of a solution of oregano oil and lactic acid (1: 1). Untreated samples were considered as control. Samples were packaged with polyethylene low density films and stored in controlled refrigeration cameras to 4ºC, for 24 days.

At different times were performed microbial counts of Total Aerobic Mesophiles, Enterobacteriaceae and *Pseudomonas sp* in specific culture media. Results were modeled mathematically using Gompertz model or linear regression. Specific growth rate (mu), Lag Phase Duration (LPD) and Maximum Population Density (MPD) derivated Gompertz parameters were determined.

Gompertz model was appropriate to quantify microbial growth in all conditions studied, with a very low percentage error (<3%). Meanwhile, *Pseudomonas sp* in treated samples showed no development, having to apply the linear regression model. The ratio of MPD Total Aerobic Mesophiles of untreated samples compared to treated ones was 1.36 times, while in the case of Enterobacteriaceae was 1.42 times.

From the results, we can infer that the combined application of these obstacles is effective to extend the useful life of this food.

Keywords: beef, UVC light, essential oils, mathematical models

[P.002]

Effect of HHP (High Hydrostatic Pressure) treatment on microbial behaviour and shelf-life of cape gooseberry pulp

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The use of High Hydrostatic Pressures for food processing have been increased during the last decade, since this technology is considered as non-thermal and allows to obtain processed food with an outstanding overall quality. The fruit of *physalis peruviana* contains considerable amounts of vitamins A and C, moreover their high content in minerals such as phosphorous, iron, potassium and zinc. Besides *physalis peruviana* fruit is considered as a good source of substances with antioxidant capacity.

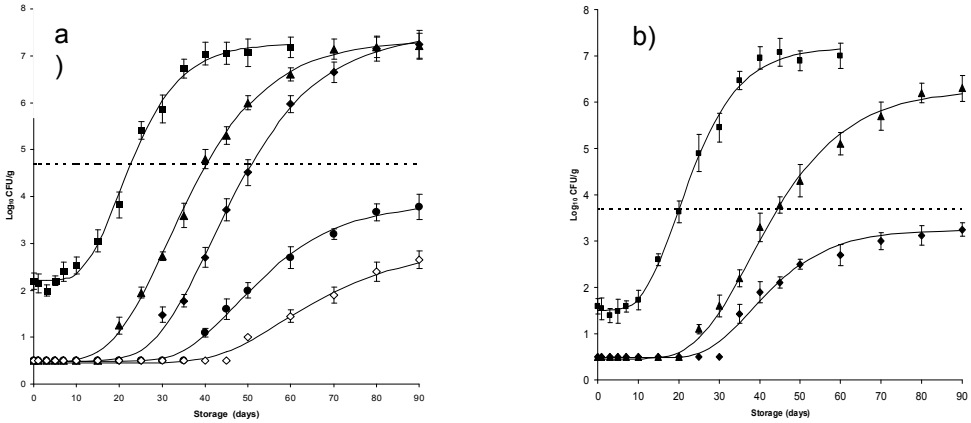
The aim of this work is to study the effect of the application of high hydrostatic pressures on microbiology and shelf life of pulp from *physalis peruviana*.

Samples of *physalis peruviana* pulps packed into polyethylene bags were submitted to hydrostatic pressures 300 and 400 MPa during processing times of 1,3 and 5 minutes respectively. Samples were then maintained under refrigeration temperature (4°C) during 90 days. Microbiological assays were performed to measure the evolution of aerobic mesophilic microorganisms (AMM), moulds and yeast. In order to estimate growth kinetic parameters including the shelf-life, growth curves of the experimental data obtained were fitted to the re-parameterized version of the modified Gompertz equation, as follows:

$$\log(N(t)) = \log(N_{\max}) - A \cdot \exp\left\{-\exp\left\{\left[\left(\mu_{\max} \times 2.7182\right) \cdot \frac{\lambda - \mathrm{SL}}{(A)}\right] + 1\right\}\right\} + A \cdot \exp\left\{-\exp\left\{\left[\left(\mu_{\max} \times 2.7182\right) \cdot \frac{\lambda - t}{(A)}\right] + 1\right\}\right\}$$

Where: N(t) is the viable cell concentration (log10 CFU/g) at time t. A is related to the difference between decimal logarithm of maximum bacterial growth attained at the stationary phase and decimal logarithm of the initial value of cell concentration. μ_{max} is the maximal specific growth rate (△ log10 (CFU/g)/days), λ is the lag time (days), N_{max} is the microbial threshold value (log10 CFU/g), SL is the microbiological acceptability limit (i.e., the time at which N(t) is equal to N_{max}), and t is the storage time.

After HHP treatment, plate counts of AMM, moulds and yeast experimented a significative decrease for all treatments, regarding to shelf-life, a typical development of microbial growing kinetic shapes was obtained, allowing to model data with Gompertz equation and to get characteristics kinetic parameters. Figures 1a and 1b shows growth curve of moulds and yeast and AMM respectively during 4°C storage.



Growth curve of mesophilic microorganisms, moulds and yeasts in untreated and HHP-treated gooseberry pulp during storage at 4°C for 90 days. Control (■), 300 MPa/1 min (▲), 300 MPa/3 min (◆), 300 MPa/5 min (●), 400 MPa/1 min (◇).

As conclusion HHP treatments at 300 MPa for 5 min or above, were sufficient to keep the spoilage microbiota naturally present in *physalis* pulp to undetectable levels during the whole storage period, extending the microbiological shelf-life of *physalis* pulp for more than 90 days at 4°C. HHP technology seems to be a good option for microbiological stabilization of *physalis* pulp and it may offer a promising alternative to conventional thermal treatment.

Keywords: physalis peruviana, Gompertz equation, High Hydrostatic Pressure, shelf-life

[P.003]

Direct dynamic analysis, model development, and Monte Carlo simulation of growth of C. perfringens in cooked beef during cooling

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Clostridium perfringens is a spore-forming anaerobic pathogen that can be found in cooked meats regulated by the USDA Food Safety and Inspection Service. *C. perfringens* can cause acute abdominal pain, stomach cramps, and diarrhea in consumers, and is one of the most common causes of foodborne illness in the United States. The spores of *C. perfringens* are distributed ubiquitously in the environment and will survive the temperatures normally used to cook meat products. It is one of the most rapid growing foodborne pathogens. Rapid cooling is critical to limit the germination of the spores and prevent the growth of this microorganism.

The objective of this study was to develop a new dynamic method to directly construct a tertiary model for predicting the growth of *C. perfringens* in cooked beef. A numerical algorithm was developed to simultaneously solve the primary and secondary equations used to simulate the bacterial growth under dynamic conditions. Multiple dynamic growth curves obtained under different conditions were used to develop kinetic models during data analysis. A bootstrap method was used to calculate the 95% confidence intervals of kinetic parameters.

The kinetic models were validated using the growth curves not used in model development. The validation results showed the predictions agreed well with the experimental observations. The mean residual of predictions (RP) was -0.02 ± 0.23 log CFU/g. The RPs were < 0.4 log CFU/g for relative growths < 1 log CFU/g. Overall, 74% of the RPs were < 0.2 log CFU/g, 7.7% > 0.4 log CFU/g, while only 1.5% > 0.8 log CFU/g. Further, the dynamic model also accurately predicted isothermal growth curves arbitrarily chosen from the literature. Finally, Monte Carlo simulation was used to calculate the probability of > 1 and 2 log CFU/g relative growth at the end of cooling.

This study provides a new probabilistic approach to estimate of the growth of *C. perfringens* in cooked meat during cooling. The results of this study can be used by the food industry and regulatory agencies to assess the safety of cooked beef in the event of cooling deviation.

Keywords: Clostridium perfringens, dynamic modeling, Monte Carlo simulation

[P.004]

The USDA integrated pathogen modeling program

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The USDA Integrated Pathogen Modeling Program developed in 2013 (IPMP 2013) is a user-friendly comprehensive data analysis and curve-fitting tool for application in predictive microbiology research and education. This tool is designed with interactive user interfaces that guide the users through different steps of data analysis, allowing the users, without the need of programming, to analyze experimental kinetic data of both primary and secondary models. It was developed as a free alternative to SAS®, R, or other more sophisticated statistical packages for model development in predictive microbiology.

Several primary and secondary models commonly used in predictive microbiology are included in the software package. The models include a three-parameter logistic model for growth curves without lag phases, reduced Huang and Baranyi models for growth curves without stationary phases, growth models for complete growth curves (Huang, Baranyi, and re-parameterized Gompertz models), survival models (linear, re-parameterized Gompertz, and Weibull models), and secondary models (Ratkowsky Square-root, Huang Square-root, Cardinal, and Arrhenius-type models). Results of comparative analysis showed that the accuracy of IPMP 2013 is equivalent to those obtained from SAS® or R.

Since the last release, new functions have been added to IPMP 2013. IPMP-Global Fit is a companion product designed to minimize global errors by simultaneously analyzing kinetic curves collected under different conditions. IPMP-Dynamic Prediction is another extension of IPMP 2013 for prediction of microbial growth under dynamic conditions. These tools can be used by the scientists in the food industry, regulatory agencies, and universities to extend the research and application of predictive microbiology.

Keywords: Modeling, Software, Simulation

[P.005]

Computer simulation of microwave-assisted pasteurization processes

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Microwave-assisted thermal pasteurization process is a technology developed for cooking foods with extended shelf-life under refrigeration. It utilizes microwave heating in combination with hot water immersion to enhance the inactivation of both spoilage and foodborne pathogens in packaged foods. Non-proteolytic Clostridium botulinum is a major safety hazard for extended shelf life refrigerated foods.

This study was conducted to evaluate the effect of various processing parameters on the accumulation of thermal lethality in products processed in a microwave-assisted pasteurization system (MAPS). Thermal processing parameters were selected to inactivate the spores of *C. botulinum* Types B and E in 10 oz. beef meatball trays and Type E in 16 oz. salmon fillet trays. Monte Carlo simulation was used to analyze the lethalities accumulated throughout the entire MAPS.

The simulation results showed that 63-70% of the total lethality was accumulated in the microwave-assisted heating (MAH) section. The remaining lethality was accumulated in the cooling section, suggesting that the cooling section can contribute a significant portion of the lethality. With a target lethality of 6 log-reductions in the spores, the simulation results showed that more than 98.8% of the processes will achieve a minimum of a 5-log reduction of the spores of *C. botulinum* Type B in 10 oz. beef meatball trays, and more 98.5% of the processes achieve > 5 log-reductions in the spores of *C. botulinum* Type E in 16 oz. salmon fillet trays.

According to the results of sensitivity analysis, the MAH section is critical to the accumulation of lethality in the products. The heating temperature, heating rate, and residence time in the MAH section are the top-three most sensitive parameters influencing the total lethality, followed by the temperature in the pre-heating section. The results of this study may be used to improve the design and operation of the MAPS.

Keywords: Monte Carlo simulation, Predictive modeling, Non-proteolytic Clostridium botulinum, Microwave-assisted pasteurization

[P.006]

The modelling for production of single cell protein from Saccharomyces cerevisiae NCYC 1530 by fermentation process

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A mathematical model was describing the kinetics of continuous production of single cell protein from date syrup as substrate. The model takes into account the substrate utilization for growth and maintenance and the effect of substrate concentration and cell death rate on the net cell growth and substrate utilization during the fermentation process. Experiment was done with Saccharomyces cerevisiae NCYC 1530 in fed-batch fermentations using three different feeding rates, with inoculum sizes 8 and 12 g/l for each feeding rate. Low feeding rate started with 6 g/h sugars and ended with 21 g/h, medium started with 11 and ended with 31.5 g/h, and high one started with 12.5 and ended with 42 g/h. The fermentation time was 7 hours, 30°C, aeration at 0.5 vvm, and stirring between 500 and 900 rpm. In the runs at low substrate feeding rate and high inoculum size (12 g/l), the final yeast biomass concentration was 17.8 g/l. Only traces of ethanol and sugars were detected in the broth at the end of fermentation and the biomass yield on sugar was 95.1% of the theoretical calculations. In case of propagation at medium substrate feeding rate (11-35.5 g sugar/h) and low inoculum size (8 g/l) the yeast biomass concentration at the end of fermentation was 16 g/l. Propagation at medium substrate feeding rate and high inoculum size (12 g/l) ended with yeast dry matter concentration of 20.6 g/l. Biomass yield on sugar was 96.5% of the theoretical, indicating that the growth was fully oxidative. In the propagation at high substrate feeding rate (12.5-42 g sugar/h) and low inoculum size the maximum yeast concentration reached was 16.6 g/l. Relatively high amounts of ethanol were formed (2.6 g/l), 1.2 g/l sugar remained in the fermentation broth unconsumed, and a maximum specific growth rate of 0.20 h^{-1} was reached.

Keywords: single cell protein, Saccharomyces cerevisiae, date fruits, yeast propagation

[P.007]

A dilution method for airborne conidia of Penicillium chrysogenum to determine mould free shelf-life of food products

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Introduction: Many food products, such as bread, are subject to post contamination by fungal conidia that are present in the air. Challenge tests should be carried out to determine the time at which a colony is visible, ca 3mm diameter. Real contamination involves air-borne conidia that are present in a few number at the surface of the product. At present a large number of conidia suspended in aqueous solutions are inoculated at the surface, or in the product, thus resulting in erroneous, shorter, shelf-life of products.

Methods: In order to reproduce a real fungal contamination of food products by air borne conidia, a method to inoculate a number of conidia in the range 1-9, at the surface of agar media was developed. To summarize, the method was based on dry-harvesting the conidia in the lid by gently taping the bottom of the dishes where sporulating mycelium was grown, adsorbing the conidia on glass beads, and, transferring the beads to successive Petri dishes to "dilute" the samples.

Results: Among the eleven factors tested by means of an experimental design, the most important factors were the incubation time of the sporulating mycelium, the resting time to dislodge as many conidia as possible from the lid, the number of beads and the number of successive dishes. Decimal "dilutions" were achieved by transferring 10 beads to the successive dishes with a mixing time of 10 sec. It was shown for *Penicillium chrysogenum* that an average of 3 colonies per dish was obtained for 3 days incubation time, 24 h resting time, 10 beads, and, the fourth dish.

Keywords: Air-borne conidia, Challenge-test, Penicillium, Experimental Design

[P.008]

Effect of alkaline pH on the heat resistance of Salmonella enteritidis and validation of model parameters in egg whites

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Egg white is widely used in food formulation for its emulsifying properties; however this ingredient may be contaminated by *Salmonella enteritidis*. To ensure food safety, heat treatment should be applied with an upper temperature of 62°C. Moreover egg white also presents the particularity to have an alkaline pH close to 9.2 which could be taken into account to optimize pasteurization process.

The aim of this study was to quantify the effect of alkaline pH on the heat resistance of *Salmonella enteritidis*.

Thermal inactivation kinetics of *Salmonella enteritidis* NCTC 13349 were performed using capillary method. Different conditions for heating and recovery media were studied from pH 7 to 10. D values are determined and the effects of the heating and recovery pHs were quantified using a modular model approach. An original methodology has been developed to validate, in food matrix, determined parameter values quantifying thermal bacterial inactivation including both heat treatment and recovery conditions.

A large effect of alkaline pH of heating and recovery media was observed on heat resistance. An increase of heating medium pH from 7 to 9.5 leads to a five fold reduction the *Salmonella enteritidis* NCTC 13349 heat resistance. Whereas an increase from pH7 to pH 9.5 of the recovery medium pH leads to a two fold reductions of heat treatment time for the same efficiency. Parameter values has been validate in egg whites.

Taking into account the alkaline pH of egg white in pasteurization process allows a reduction of heat treatment and efficient bacterial load reduction. These results ensure a risk/benefit approach yielding destruction of *Salmonella enteritidis* and functional properties of egg whites.

Keywords: thermal inactivation, alkaline pH, validation

[P.009]

Thermal inactivation of Salmonella Typhimurium and Escherichia coli K 12 in milk powder

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Introduction: During processing milk powders are subject to contamination by pathogens such as *Salmonella* Typhimurium and *Escherichia coli*. Thermal inactivation is a well documented method to inactivate microorganisms in humid products, however less is known about decontamination of dry products.

Methods: Bacterial suspensions were mixed with milk powder and dried overnight by placing the mixtures in equilibrium with LiCl, KCH_3COOH, KCO_3, and $MgNO_3$ to obtain 0.11, 0.25, 0.44 and 0.58 a_w. Thereafter the contaminated products were treated at 85, 90, 95, and 100°C for 0-4 min. Inactivation was modelled by first order and Weibull models.

Results: During drying, less than one log decrease in viability was observed for the two microorganisms. A treatment of *S.* Typhimurium at 100°C for 2 min exhibited a greater inactivation at 0.44 a_w (5.16 log UFC/g reduction) than at the other water activities. It was shown that thermal destruction of the organisms could not be described by first order kinetics. *S.* Typhimurium was less resistant than *E.coli*. The parameters, α and β, of the Weibull model were determined for each temperature, and the effects of water activity of the milk powders on α and β were evaluated.

Discussion: This study enables the determination of protocols for decontamination of milk powders as a function of water activity. Some more studies are needed to quantify the effects of the distribution and the size of the particles of milk.

Keywords: Thermal inactivation, Water activity, Milk powder, Pathogens

[P.010]

F-value calculator - A tool for calculation of acceptable F-value in canned meat reduced in NaCl

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Canned meat products are usually protected against growth of C. botulinum by use of combinations of heat, sodium chloride and sodium nitrite. However, during the last decade, a request from customers and food authorities to reduce the amount of sodium chloride in meat products have arisen due to the negative health effects of sodium chloride. The UK authorities have set new targets for maximum levels of sodium chloride in canned meat at less than 2.0%. In some cases such levels of sodium chloride equal less than 2.5% aqueous salt and thus Codex Alimentarius guidelines recommend a heat treatment at least equal to $F_{121.1}$ = 3.0. This is a rather high heat treatment that may affect the eating quality of the meat product negatively. Thus, we investigated the possibility of applying a lower heat treatment without affecting the microbial safety regarding sporulation and growth of C. botulinum.

Canned meat products having aqueous salt from 1.7 to 3.5% were spiked with gas producing Clostridia and autoclaved at F values from 0.5 to 3.3 and subsequently stored at 37°C. The cans were regularly inspected for blown cans indicative of Clostridia growth until all the cans were blown. By fitting corresponding values of time and % blown cans (MicroFit 1.0), the time until 50% of the cans were blown ($bomb_{50}$) was estimated as a measure for “relative shelf life”. Subsequently corresponding values of aqueous salt, F-value and “$bomb_{50}$” were used to develop the “F-value calculator tool”.

From input of a known satisfactory combination of actual aqueous salt and the actual F value, the tool calculates the new acceptable F- value giving the same protection for a similar product, with a requested, reduced level of aqueous salt. The F value calculator tool is available at www.dmripredict.dk

Keywords: Canned meat products, C. botulinum, Reduced sodium chloride, Equivalent F-value

[P.011]

Impact of the fat on the apparent heat resistance of lactic acid bacteria in food

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In refrigerated pasteurized products, Lactic Acid Bacteria (LAB, defined mainly as the flora capable of developing on the MRS agar) are the microflora which influences the setting of the product Shelf Life (SL) due to organoleptic degradation. Likewise, LAB are also the main flora limiting the SL of refrigerated pasteurized foie gras (duck liver). A better knowledge of this flora is then essential to enable an optimization of the process while guaranteeing a high standard quality of the product. In particular, values of heat resistance found in the literature are too low to explain the presence of LAB in product after application of industrial heat-treatment conditions.

The objective of this study was then to investigate the impact of the fat on the heat resistance of LAB in food. That was done by setting two successive experimental designs. All the results were quantitatively analyzed (Weibull model, ANOVA, secondary model).

First, seven factors associated with the characteristics of growth prior to heat treatment (medium, growth phase, temperature), the resistance during the treatment itself (media and heat treatment conditions) and the recovery media post-treatment, were studied in a fractional factorial design (2^{7-2} = 32 conditions). The results enhanced a very strong influence of the foie gras matrix on LAB apparent heat resistance, both during the treatment and the recovery, with an interactive effect of the heat-treatment temperature.

Next, to investigate more in details the protective effect of the foie gras matrix, the apparent heat resistance of LAB was studied in a Latin Square design, set at 5 levels, with heat-treatment temperature, percentage of fat in heat-treatment media and in recovery media as factors (25 conditions). These results enable a better understanding of the protective phenomena of fat on LAB in foie gras and more generally in fatty food product.

Keywords: Lactic acid bacteria, Fat, Heat resistance, Recovery

[P.012]

Predicting the behaviour of Yersinia enterocolitica and Listeria monocytogenes in Italian style fresh sausages under dynamic growth-death conditions

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Italian style fresh sausage is a traditional pork product that is frequently consumed raw, usually after drying. So far, the prediction of the behaviour of microorganisms in varying environments has been carried out through separate growth or death models. The aim of this study is to define a predictive model able to describe the kinetics of *Yersinia enterocolitica* and *Listeria monocytogenes* in dynamically changing growth-supporting and inactivating environments.

Sausages were separately inoculated with *Yersinia enterocolitica* and *Listeria monocytogenes* and stored for 480 hours at 8, 12, 18 and 20°C. Other than detection and enumeration of *Yersinia enterocolitica* and *Listeria monocytogenes*, also lactic acid bacteria, pH and water activity (a_w) were checked. The pH was fairly constant, while the a_w changed dynamically, being the primary cause of shifting from growth to death behaviour.

The water activity took up values from growth (R_G), uncertain (R_0) and death (R_D) regions, the first being R_G=[0.92, 1] for *Listeria monocytogenes* and [0.95, 1] for *Yersinia enterocolitica*. The width of R_0 was assumed to be 0.03 for both microorganisms. In this region, the dynamics were considered unpredictable, and for the sake of simplicity, neither growth nor death was predicted. The effect of acid lactic bacteria on growth modified the growth rate.

The predictions were based on data gained in static environments and on the dynamic model of Baranyi and Roberts (1994). For numerical reasons, a rescaled version of a_w was introduced according to Gibson et al. (1998). Predicted growth-death curves obtained from the model were compared to challenge test results and they were found satisfactory at the storing temperatures considered.

Our approach can be used to predict bacterial growth/death kinetics under temporal variation of storage environments, which is vital when assessing the safety of fresh sausage. We envisage similar applications to other RTE meat products, too.

Keywords: Listeria, Yersinia, Sausage, Dynamic

[P.013]

Precision food processing: Combining microbial inactivation and quality deterioration models for fruit juice safety and quality

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Models predicting the decimal reduction times (*D* values) of non-stressed and acid-stressed *E. coli* O157:H7 were established in simulated fruit juices with varying pH and soluble solids (SS), at different heating temperatures. Generally, acid stress exposure increased the thermal resistance of the challenge organism. The *D* values of non-stressed cells fitted into a reduced 2-factor interaction model, with temperature, pH and pH×SS as significant predictors. On the other hand, the *D* values of acid-stressed cells fitted into a quadratic model with pH, SS, pH^2, and SS^2 as significant predictors. Furthermore, *D* value ratios demonstrated the possible cancellation of acid stress-induced thermal resistance when the suspending medium SS >55 °C. Validation showed acceptable predictive efficacies of both inactivation models. Together with fruit juice physicochemical factors (pH, SS, dilution rate, initial ascorbic acid content) and heating temperature, the *D* value ranges obtained in non-stressed and acid-stressed *E. coli* O157:H7 were then used as predictors in models for deteriorations of quality attributes in heat-treated simulated and real citrus juices, including ascorbic acid content and Commission Internationale de l'Éclairage (CIE) color space parameters. Significant predictive models, validated to have acceptable predictive efficacies were established for estimating degradation of ascorbic acid in simulated fruit juices subjected to unique thermal process schedules. On the other hand, individual models for the changes in measured color parameters CIE *L** (lightness-darkness), *a** (redness-greenness), *b** (yellowness-blueness) were established and validated for real citrus juices, including Philippine native orange, native lemon, orange, and grapefruit. Our group is currently establishing models for the consumer acceptability of the test citrus juice color using the same physicochemical and process parameters as predictors. So far, the results of these works demonstrate the potential of combining microbial inactivation and quality deterioration models for a more precise, effective, and comprehensive control of safety and quality of heat-treated fruit juices.

Keywords: Microbial inactivation model, Quality deterioration model, Ascorbic acid degradation, Color changes

[P.014]

Survival kinetics of Salmonella and enterohemorrhagic Escherichia coli under low water activity environments and on low water activity foods

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Food poisoning induced by pathogenic bacteria occasionally occurs through the ingestion of low water activity (a_w) foods such as nuts, chocolate, hard cheeses, etc. *Salmonella enteric* and pathogenic *Escherichia coli* are highly tolerant to desiccation compared with other bacterial species. Modelling the survival kinetics of both bacterial species as a function of a_w and temperature could be useful for microbial risk assessments for these bacteria. In this study, we aimed to develop predictive models that can be used to estimate the number of bacteria in low a_w environmental conditions and low a_w foods. We investigated the survival kinetics of 4 serotypes of *Salmonella enterica* (Stanley, Typhimurium, Chester, and Oranienburg) and 3 serotypes of pathogenic *Escherichia coli* (O26, O111, and O157) when exposed to a combination of three temperatures (5°C, 15°C, and 25°C) and five a_w conditions (0.22, 0.43, 0.58, 0.68, and 0.93). The bacterial cultures (20 µl) were placed onto sterile plastic plates and dried in a safety cabinet for 4 hours. After drying, the plates were placed in plastic containers that were adjusted to each a_w condition using saturated salt solutions. In addition to these *in vitro* experiments, we examined the survival kinetics of these bacterial species on the surfaces of chocolate, cheddar cheese, almonds, and radish sprout seeds under various temperatures. Regardless of the a_w and the serotype, a rapid decrease in the number of viable bacterial cells was observed at 25°C compared with 5°C. The survival kinetics was successfully described by Weibull model. The estimated Weibullian model parameters were described as a function of temperature but not of a_w. The results of this study enable the prediction of the number of pathogenic bacteria in low a_w foods under various storage temperatures. This information could contribute to assessing the risk of these bacteria in low a_w foods.

Keywords: low water activity, survival kinetics, Salmonella, enterohemorrhagic Escherichia coli

[P.015]

Quantitative analysis of differential gene expression in RNA-sequencing data for the investigation of the bioprotective effect of Lactobacillus sakei in beef carpaccios

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Aiming at better understanding the mechanism of the biopreservative effect of a four-strain cocktail of *Lactobacillus sakei* in limiting beef carpaccio spoilage, we analyzed gene expression of the cocktail as a function of spoilage occurrence.

Naturally contaminated batches of beef carpaccios were surface-inoculated at 10^4 cfu/g with an in-house cocktail of four *L. sakei* strains selected for their combined bioprotective properties and whose whole genome sequence was established. Carpaccios were stored at 8°C during 6 days before collecting bacterial nucleic acids. Spoilage occurrence was visually assessed by color change from red to brown. Gene expression of the cocktail was performed by a RNA-seq-based transcriptomic analysis. To quantify the differential expression of *L. sakei* genes as a function of spoilage occurrence, sequence count data were analysed with a negative binomial using the DESeq Package (R software).

The differential gene expression analysis of the four *L. sakei* bioprotective strains revealed that a panel of genes or transcripts were differentially expressed between spoiled and not spoiled carpaccios. The main functions associated to these genes were correlated with energy metabolism and oxidative stress.

This study provides new insights into the bacterial functions involved in food biopreservation.

Keywords: spoilage prediction, RNA-seq, bacterial functions/interactions, biopreservation

[P.016]

Survival of pathogenic microorganisms in spices and herbs

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Spices and dried aromatic herbs can be cultured where hygiene conditions might be poorly controlled and products can have high levels of spoilage and pathogenic microorganisms. Since spices and dried herbs are commodities with low water activity they are usually stored at room temperature under dry conditions. Hence they have a shelf-life of 2-3 years.

Although drying can inhibit microorganism growth, it may not completely inactivate pathogens. Thus the purpose of this study was to investigate survival of pathogens during storage of spices and dried herbs.

A meta-analysis was performed on the available published data to identify the most critical factors that influence survival in spices and dried herbs. This showed that Gram positive bacteria (spores and sporeforming cells) have lower inactivation rates than Gram negatives. In addition, important factors of variability for Gram positive bacteria were found to be decontamination treatment before storage and product type, while for Gram negative only product type was identified as relevant factor.

From studies available on *Salmonella* spp. it was concluded that water activity plays also a significant role in survival. Additionally, survival of *Salmonella* Infantis and *Listeria monocytogenes* was experimentally monitored in powdered paprika under regulated storage conditions. The obtained results were fitted with log-linear, Weibull, biphasic-linear and Geeraerd models. *Salmonella* spp. had significantly lower inactivation rate than *Listeria monocytogenes*. Nevertheless, it was found that pathogen survival curves are best described with the Weibull model for *Salmonella* Infantis and the biphasic-linear model for *L. monocytogenes*, pointing to a more pronounced reduction at the first phase of storage.

In conclusion, the reduction of pathogens during storage of spices and herbs might be limited depending on the type of organism present. Control of the initial levels of microbial contaminants is therefore of key importance.

Keywords: spices, herbs, storage, inactivation

[P.017]

Predicting safe sandwich production

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Time and temperature control is crucial to avoid growth of pathogens during production and serving of cold ready-to-eat meals. The Danish guidelines state that chilled foods, such as sandwiches, should not be outside the cold chain for more than 3 hours including the time for preparation and serving. However, Danish sandwich producing companies find it challenging to comply with this and have expressed a need for more flexibility. The Danish guidelines do allow for a prolongation of the acceptable time outside the cold chain, if the safety of the specific production can be documented. There is, therefore, room for developing targeted tools for evaluating the time-temperature scenarios in sandwich production.

This study describes a decision support tool developed to offer the producers more flexibility. Based on time/temperature measurements obtained during preparation combined with information on the prehistory of ingredients and the expected time/temperature conditions of distribution and serving, the potential growth of *Listeria monocytogenes*, *Salmonella* and psychrotrophic *Clostridium botulinum* in the sandwiches is predicted. Applying the lag times of these pathogens as the critical limit, the tool determines if the sandwich production is safe by evaluating whether any of the lag times have been exceeded during the total preparation, distribution, and serving time.

The growth models employed were built as part of the study using a “worst case” ingredient, cooked, sliced chicken breast. Validation was performed at dynamic temperature profiles which were derived from observational studies of sandwich production practices in Danish companies.

Keywords: Listeria monocytogenes, Salmonella, Clostridium botulinum, Relative lag time

[P.018]

Growth kinetics parameters of Salmonella spp. in the peel and in the pulp of custard apple (Annona squamosa)

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Introduction: Data from epidemiological investigations indicate that *Salmonella* is the main etiological agent associated with fruit-borne disease outbreaks. Custard apple (*Annona squamosa*) is a tropical fruit produced in Brazil, whose consumption and exportations have increased due to its exotic flavor, richness in nutrients, antioxidant compounds and consumers' search for healthier foods. Despite this, no data on the behavior of *Salmonella* in this fruit are found in the literature. Thus, the purpose of this study was to determine the growth kinetic parameters (maximum growth rate, lag time) of *Salmonella* spp. on the peel and in the pulp custard apple at 10, 15, 20 and 30 °C.

Methods: Samples of peel and pulp of custard apple were inoculated with a cocktail of three strains of *Salmonella* spp. (*S*. Typhimurium, *S*. Enteretidis and *S*. Montevideo) (10^2–10^3 CFU/g) and further stored at 10, 15, 20 and 30ºC. Sampling was carried out at different intervals and count was done using MLCB agar incubated at 37°C/ 24 h. The growth kinetic parameters were obtained by fitting the experimental data to Baranyi model using the DMFit software.

Results: The growth data indicated that *Salmonella* can survive and multiply in both, the peel and in the pulp of the custard apple. Lower temperatures retard, but do not prevent *Salmonella* growth. The growth kinetic parameters and the influence of temperature variation on the primary parameters will be presented and discussed in terms of impacts for custard apple safety.

Discussion: The results of this study show that tropical fruits such as custard apple comprise substrates that allow the growth of pathogens such as *Salmonella*, both in the pulp and in the peel. The main implications are that even contamination of peel at pre- or post-harvest steps may lead to spread of *Salmonella*, which may potentially affect public health.

Keywords: Predictive microbiology, Salmonella, custard apple

[P.019]

Modeling bacterial growth on sushi (hosomaki) exposed to different temperatures

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The consumption of oriental foods is increasing worldwide and foodborne diseases have been reported after the consumption of sushi. In Brazil, sushi have been commercialized refrigerated, however different producers declare that refrigeration is not necessary because sushi usually present low pH. The objective of this work was to model the growth of *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* in sushi (Hosomaki) with pH 4.2 exposed to different temperatures.

Microorganisms were inoculated separately on sushis that were stored at 7 and 25°C. Microbial analyses were carried out in varied time intervals. Growth curves were built by fitting the data to the Baranyi's DMFit model. The pH was measured at each sampling time.

Results demonstrated that sushis exposed to 25°C inhibited and reduced the growth of *B. cereus,* during 34 h, inicial counts were reduced approximately 2 log. Log phase was not observed in the growth of *E. coli* and *S. aureus* strains at 25°C. Under 7°C, 1.0 log reduction of *E. coli* was observed after 30 h. *B. cereus* and *S. aureus* did not grow at 7°C. The pH of sushi samples remained constant at 4.2 throughout the experiment.

In Conclusion, sushi with pH 4.2 exposed to 25°C did not support the growth of *B. cereus*, *S. aureus,* and *E. coli* and was able to reduce *B. cereus* counts. At 7°C, the microorganisms did not grow and *E. coli* population was reduced in 1.0 log. This is a preliminary study and more data will be collected to improve the developed primary models and to construct a secondary model to assess the growth of *B. cereus, E. coli* and *S. aureus* on sushi under various temperatures.

Keywords: Sushi, Bacterial Growth, Modeling, Different Temperatures

[P.020]

Effect of pomegranate powder (ellagic acid) on the thermal resistance of Escherichia coli O157:H7 in ground chicken

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Health concerns have led to a search for natural plant-based antimicrobials. Ellagic acid has been shown to have antimicrobial activity against foodborne pathogens. The objective of this study was to assess the effect of pomegranate-extracted ellagic acid on the heat resistance of Escherichia coli O157:H7 in ground chicken. A full 24 factorial design was used, consisting of temperature treatment with four levels (55.0, 57.5, 60.0 and 62.5°C) and pomegranate with four levels (0.0, 1.0, 2.0 and 3.0 wt/wt% containing 70% ellagic acid). Experiments were conducted twice, providing a total of 32 survival curves. Survival kinetics was described by a three-parameter Weibull primary model. Secondary models were then developed to estimate the shape parameter β (i.e., curvature representing cells' susceptibility to stress), scale parameter γ (i.e., time to reach the first decimal reduction) and the 5.0-log lethality time t5.0 (i.e., time to reach a 5.0-log reduction), all as polynomial functions of temperature and pomegranate powder concentration. The positive effect of pomegranate concentration on both β and γ demonstrated that the phenolic compound causes E. coli O157:H7 cells to become more susceptible to heat, increasing the steepness and concavity of the isothermal survival curves. It was estimated that the 5.0-log reduction time would reach a minimum at a pomegranate powder concentration of 2.5%, producing a 50% decrease in lethality time, in comparison to that when no pomegranate powder is added. Nonetheless, a mixed-effect omnibus regression further confirmed that the greatest difference in the thermal resistance of E. coli O157:H7 happened between not adding and adding pomegranate powder. In fact, adding more than 1.0% pomegranate powder, at a constant temperature, resulted only in a marginal decrease in thermal resistance. Meat processors can use the model to design lethality treatments in order to achieve specific reductions of E. coli O157:H7 in ground chicken.

Keywords: Escherichia coli, Weibull, polynomial, predictive model

[P.021]

Contribution of Enterobacteriaceae to sensory characteristics in soft cheeses made of raw milk

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Bacteria contribute to different flavors and textures in cheeses during ripening. Most of the studies that compare flavor compounds and bacteria in cheese uses chemical analytical instruments such as gas chromatograph and mass spectrometer. In contrast, our study is based on quantitative descriptive sensory results where humans are used as analytical instruments.

Microbiological and sensory methods, namely plate counting and quantitative descriptive analyses, were used to analyze 22 soft cheeses, of which 20 were made of raw milk. Regression and correlation analysis were performed to examine possible relations between the levels of bacteria and the intensity of sensory characteristics.

Moderate correlations (r-value 0.6 and p-value <0.01) were found between the levels of *Enterobacteriaceae* 37°C and the intensity of the sensory characteristics “bitter” and “metallic”. A negative moderate correlation (r-value -0.6 and p-value <0.01) were found between the level of *Enterobacteriaceae* 37°C and the intensity of the sensory characteristic “sweet”. Moderate correlations (r-value 0.5 and p-value <0.05) were also found between the levels of *Enterobacteriaceae* 37°C and the intensity of the sensory characteristics “pungent”, “manure” and “ammonia”. Furthermore, 20 isolates of *Enterobacteriaceae* 37°C and 20 isolates of *Enterobacteriaceae* 44°C from four of the 20 soft cheeses made of raw milk were identified using api 20 E strips. Three species gave “acceptable” to “excellent” identification, namely *Hafnia alvei, Escherichia coli* and *Klebsiella pneumoniae.* It was not possible to see whether there were any different sensory profiles between these *Enterobacteriaceae* species.

The present study indicates that it is possible to predict high levels of *Enterobacteriaceae* in soft cheeses made of raw milk using only the human senses (odor and taste). Further studies with different combinations of *Enterobacteriaceae* species and mixtures would need to be evaluated to ensure comparable results.

Keywords: Soft cheeses, Pasteurisation, Sensory profile, Enterobacteriaceae

[P.022]
ISFSFP

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Abstract

Effective approaches to the solution of tasks for the safeguarding of food production based on a systematic approach, artificial intelligence methods and modern computer systems, as well as international safety standards for food production safety were researched and proposed. A methodology for the creation of intelligent systems for the solution of the task of ensuring food production safety was proposed. Means of implementing the intelligent systems for safeguarding food production were considered. A structure for an intelligent system for decision making for ensuring food production safety was proposed and its main components described.

Manufacturing situations in the food industry were formalised, and formulae and methods for the solution of the task of quality control and production safety were formulated. During the formalising and solution of the tasks of quality control and production safety, the ideas of compromise decision making schemes (Paretto Opimisation, main criterium method) which have been modified for use in a fuzzy environment were used. The peculiarity and originality of the proposed methods for the solution of tasks, lie in the fact that, due to the conservation and full use of fuzzy input data, the problem of multi-criteriality and uncertainty is effectively solved.

Key words: intelligent systems, food production safety, Paretto optimisation, multi-criterial optimisation, fuzzy data, fuzzy set methods.

Introduction

The appearance of computer systems which are highly productive and have a large memory, the fact that it is necessary to process significantly large volumes of data, the use of knowledge bases and the requirement to facilitate working with the computer by using natural language, have led to the formation of various intelligent systems (intelligent data retrieval systems, intelligent application programme packages, expert systems, intelligent decision making systems etc.) [1–5].

At the present time, one of the pressing issues in food production is that of ensuring production safety. In the Republic of Kazakhstan, as in other countries, legislation has been passed [6], which provides a legal basis for the safeguarding of food production for the protection of human life and health, the legal interests of the consumer and protection of the environment.

In food production, special systems are being developed and introduced for the analysis of hazards and the determination of critical points of control for the systematic identification, evaluation and control of hazards which influence the safety of production in the whole production chain by means of defining and evaluating potential risks which are critical for food production safety, in the establishment of continual control at critical control points [7].

Food production safety is the absence of unacceptable risk, in all processes of the processing, production (preparation), handling, utilisation and elimination of food products, which could lead to harm to the life or health of an individual or to an infringement of the legal rights of the consumer, taking into account the combination of the likelihood of a hazard arising and the severity of its consequences [8].

The main objectives of state regulation in the sphere of food production safety are: ensuring the safety of food production in relation to the life and health of consumers and the environment; the protection of the legal rights of the consumer, or the environment and of national security [9].

Food production safety is ensured by the following means:

- adherence to individual requirements as established by food production safety legislation;

- verification of accordance to food production requirements as established by legislation for technical regulation, and the implementation of health and disease control and veterinary sanitary examination;

- the development and implementation of computer and intelligent systems in order to ensure food production safety.

Ensuring food production safety is one of the most important strategic tasks in any country, including the Republic of Kazakhstan. The health of the nation depends to a great extent on food production safety. Furthermore, food production safety is one of the mandatory requirements for food suppliers in developed countries. As the educational and economic level of the population rises, internal consumer demands for food production safety are also growing.

In order to show that there are issues in the area of food production safety, events which periodically occur in our country can be pointed out. For example, in March 2007, at the GATE shift camp (Atyrau Region) several hundred employees fell victim to food poisoning in the canteen run by the Turkish Company ACB Catering. It goes without saying that such food poisoning emergencies cannot be concealed, receive wide publicity and are then investigated. It is far more difficult to tackle food products which are continually harming the health of individuals a little at a time. Such harm often goes unnoticed, although the consequences may be significant. In such cases, food safety is a question of state importance which can be successfully resolved only on a systematic basis, using modern scientific methods and information technology systems. In this connection, the creation and implementation of intelligent systems to ensure food safety is an urgent security issue for any state.

Research Methods and Results

The legal basis for ensuring food production safety has been established in our country. From the 1st of January 2008, the Law of the Republic of Kazakhstan “On Food Production Safety”, which establishes the main responsibilities, came into force. [6]. In implementation, a strategic mistake would be to rely only on the punitive actions of the executive authorities. Such actions are only necessary for unscrupulous food suppliers, whereas for the majority of organisations a different approach is required, based on the implementation of international standards, and the development and implementation of intelligent systems in order to ensure food production safety.

International standards for food production safety require the implementation of a systematic approach, with various intelligent systems, supposing that the food producer will voluntarily analyse the risks and implement appropriate programmes and systems in order to warn of hazards at critical control points, which is known as an HACCP (Hazard Analysis and Critical Control Points) plan.

Since 2005, the new international standard ISO 22000 “Systems for the Management of Food Production Risk. Requirements for every Organisation in the Food Chain” has been in force, which was drawn up in order to harmonise the requirements of the CODEX ALIMENTARIUS international standards and national requirements, such as in the sphere of sanitary and hygiene regulations. This standard, together with other international standards ISO 22004 “Systems for the Management of Food Production Risk, Guidelines for the

Implementation of ISO 22000:2005" contains the requirements and recommendations in relation to the implementation of management systems for food production safety.

In the design and construction of intelligent systems for ensuring food production safety, various general scientific and statistical methods are used: artificial intelligence methods, fuzzy set theory methods, mathematical modeling and identification methods, theory and methods of multi-criterial optimisation and decision making, cluster analysis methods, multi-factorial correlation regression analysis methods, systematic analysis methods, and models and methods for the representation of knowledge [10–15]. In order to process practical and experimental data, modern computer systems and programme application packages Statistica 5.5 [16], Regress and MatLab are used.

In the formulation and solution of the task at hand, in a fuzzy environment it becomes necessary to use the knowledge and judgments of the person making decisions (DM), experts and specialists, which have a qualitative nature. In order to solve such fuzzy decision making tasks, it is necessary to include elements of intelligence in the system which is created, thus allowing for communication with the system using natural or professionally oriented language. This is made possible, based on methods of artificial intelligence, and the inclusion in the make up of the information system of the following components: a logical inference and selection choice explanation unit; algorithms for multi-criterial fuzzy optimisation; an intelligent interface.

Such systems, based on the knowledge of experts and specialists, and formed using artificial intelligence methods and fuzzy set theory, are known as intelligent decision making systems (IDMS).

According to the results of the research which has been carried out, we have identified the following steps for the creation of intelligent systems for the task of ensuring food production safety [4]:

1. The identification of the problem area and the tasks to be solved, including the comprehensive formulation of the optimisation task for effective decision making;
2. Formalisation of the knowledge of the DM, experts and specialists concerning the task at hand;
3. Forming of a knowledge and data base;
4. Development of a package of models of the object and processes being researched;
5. Construction of algorithms for the tasks being solved, for example, for ensuring food production safety;
6. Development of an intelligent user interface;
7. Programming of the models and algorithms which have been developed.

Let us further clarify several of the steps which have been listed for the method for the creation of intelligent systems for the solution of tasks in order to ensure the quality and safety of food production.

In the first step, the object under research is localised, and its basic composition is determined and the production is described, and the problem which is being resolved is comprehensively formulated.

In the second step, the expert evaluation procedures are organised [17–21], the results of which are used to formulate the task of the optimal selection of a decision according to the given criteria. In so doing, expert evaluation methods, which have been modified for cases where the input data is fuzzy in nature, are used.

Based on the formalisation of the knowledge in *step three* the structure is determined and a knowledge base is formed. The knowledge base is populated based on statistical data concerning the functioning of the object.

In the fourth step, models of the elements and sub-systems of the manufacturing facilities are developed, which are then combined into a complete package. Some or all of the models may be fuzzy. In order to construct such models, special algorithms for the synthesis of mathematical models based on fuzzy input data can be used [6].

Complex algorithms, which include within them optimisation algorithms in a fuzzy environment and algorithms for the solution of multi-criterial tasks of fuzzy mathematical programming, can be used as algorithms for the formalisation of the tasks (*point five*).

In order to establish a convenient and intelligent interface (*point 6*), and for the programming of models and algorithms (*point 7*), modern visual programming methods are used. The structure and contents of the menu must be agreed with the user (customer).

The proposed structure of the intelligent system for decision making in order to ensure food production safety, is shown in Figure 1.

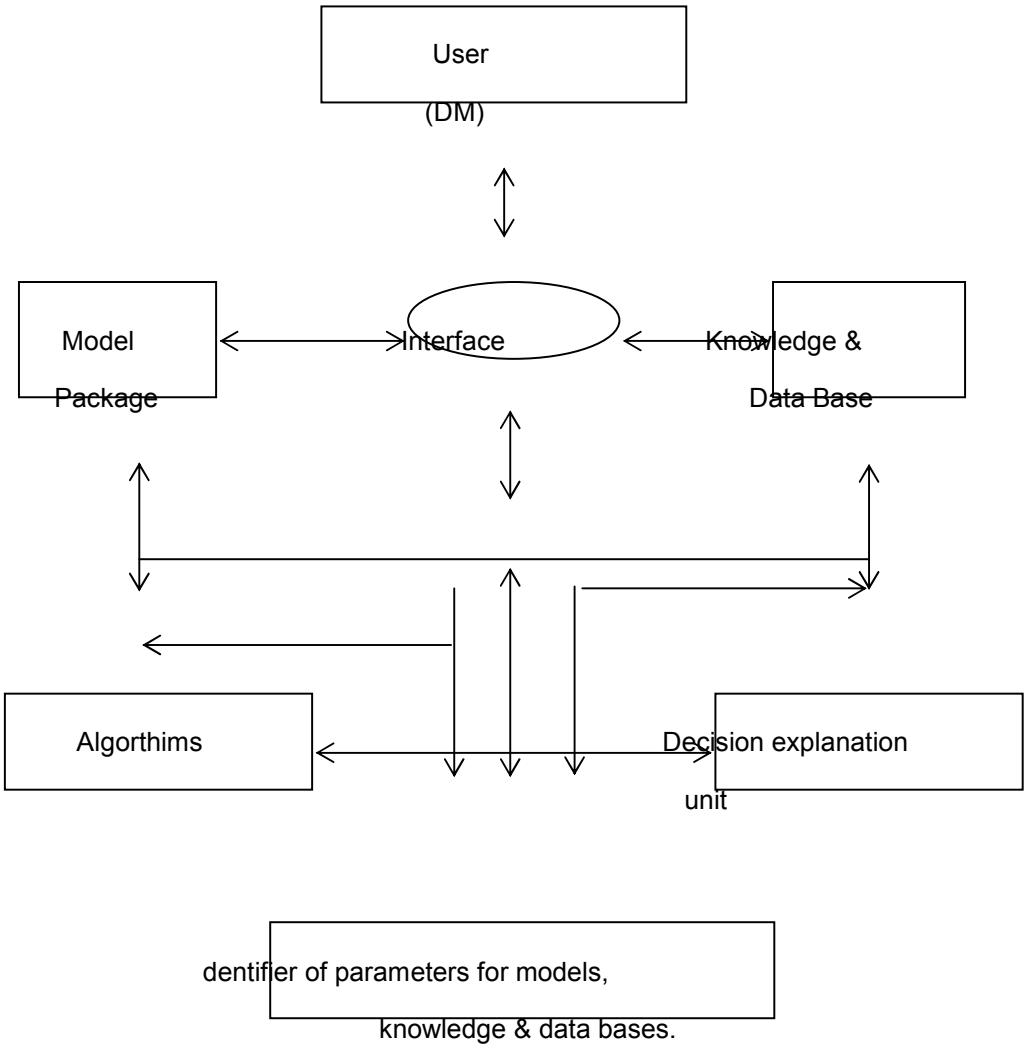

Figure 1 – Structure of the Intelligent System to Ensure Food Production Safety.

The effectiveness of the IDMS which has been developed for the solution of tasks for the ensuring of food production safety, is determined by the quality of the formulation and representation of knowledge, the models which have been developed, and the algorithms for the solution of the tasks which have been set, as well as by the convenience of the user interface.

As a result of the analysis of the current state of the problem of the monitoring and prevention of food-related risks in the Republic of Kazakhstan and in the world, it becomes clear that it is necessary to transition, in the practice of our home country, to new and effective strategies for risk control. Intensive discussion by specialists, and the wide impact on the general public related to the appearance of new or previously unconsidered risk factors in food production, have made it necessary to form new concepts of food production safety at all levels, from the field to the consumer, which can be implemented and controlled with the use of modern intelligent systems.

An evaluation of food risks as a whole is beneficial due to the transparency of the decision making process based on intelligent systems and the improvement of documentation relating to the basis for decision making. Processes for identifying dangers and determining their parameters must be considered as iterative and requiring significant improvement of the data search and the establishment of knowledge and data bases, that is require intelligent systems.

Means for the Implementation of Intelligent Systems for Ensuring Food Production Safety. IDMS are in fact hardware and software systems. Each of the functional units which has been considered can be implemented in the form of special software programmes, and the hardware is a computer, which satisfies several requirements (speed, memory, type of graphics card etc).

The software part of the IDMS is made up of programmes for the implementation of modelling tasks and multi-criterial selection of decisions in conditions of uncertainty, the fomalisation of knowledge in the form of a product, that is a programme to explain the decision making process to the user and provide information about the functioning of the system, accompanied by various tables, graphs and text messages, programmes for verifying the adequacy and adjustment of the models (identifier) and a user-friendly interface. The interface must provide the user with an interactive working regime and systems, for example, with various menus, information, prompts etc. In the future, audio features are envisaged or even dialogue in professionally oriented, almost natural language.

In order to solve the production tasks, a large volume of various types of data is stored in the knowledge base, and in order to handle this data (search, process, output, update etc) it is necessary to create a special programme, for example a knowledge and data base control system (KDBCS).

In order to effectively create the programmes, structured programming methods and special programming resources, containing editors, compilers, loaders, debugging tools etc, such as a C, Delphi or another environment, can be used, as well as specialised programming packages.

Approaches to the development of intelligent memory for the processing of fuzzy data, based on the ortho-coordinated association of intelligent memory systems with fuzzy logic [22], which may be used as a co-processor in computers for the solution of fuzzy tasks, which are difficult to formulate, are known.

Let us provide the results of the research which we have carried out for the formalisation of manufacturing situations in the food industry, which arise from the formulation and solution of tasks for the ensuring of the quality and safety of production:

1) MS 1 — the control of production is described by one criterium (objective function) and several limits, for which both the criterium and any of the limits may be fuzzy. This includes multi-criterial tasks when it is possible to select one of the criteria as an objective function (or to

carry out a contraction of the local criteria), with the remaining criteria being considered as limits;

2) MS 2 — a situation where it becomes necessary to formulate the decision making task in the presence of several criteria. Here, due to the various physical characteristics of the criteria, their contradictory nature, the fuzziness of their descriptions and other reasons, it is not possible to immediately reduce the task to one with a single criterium;

3) MS 3 — a more general situation, where the results of the functioning of the manufacturing facilities are evaluated by several criteria and limits, each of which may be fuzzy. Control of production, in this case, is reduced to the combined use of approaches developed for the previous situations.

For the MSs which have been considered, let us set out the formulation of the tasks for ensuring the quality and safety of food production and the proposed methods for their solution.

Firstly, let us give our attention to the situation where the fuzzy mathematical programming (FMP) task, is formulated for one criterium and several limits, that is according to manufacturing situation 1 (MS 1).

FMP task 1. Let there be a single normalised criterium in the form - $\mu_0(x)$ and *L* limits with fuzzy instructions $f_q(x) \tilde{\geq} b_q, q = \overline{1, L}$. We propose that the membership function for sanctifying the limits $\mu_q(x)$ for each limit, be constructed as a result of dialogue with with DM, experts and specialists. Let either the range of priorities *I*={1,…,L}, or the weighting vector $\beta = (\beta_1, ..., \beta_L)$ for the limits, reflecting the relative importance of the limits at the moment of the formulation of the optimisation task, be known.

Then the general form of the FMP task is:

$$\max_{x \in X} \mu_0(x)$$

for the conditions $f_q(x) \tilde{\geq} b_q, q = \overline{1, L}$

and can be written:

$$\max_{x \in X} \mu_0(x),$$

$$X = \{x : \arg \max_{x \in \Omega} \mu_q(x),\ q = 1, 2, ...,\ L\}$$

The given formulation of the FMP task, with a non-fuzzy objective function and fuzzy limits with fuzzy instructions, is represented as the attempt to maximise the objective function and fully satisfy the requirements of the limits. If it is accepted that all the membership functions are Gaussian, then the formulation of the FMP tasks has the form:

$$\max_{x \in X} \mu_0(x),$$

$$X = \{x : x \in \Omega \wedge \mu_q(x) = 1,\ q = 1,2,..., L\}$$

We have derived the non-fuzzy mathematical programming task with maximised objective function for the non-fuzzy set *X*. Further we will propose concavity of the objective function

$\mu_0(x)$, limit $\mu_q(x),\ q = \overline{1,L}$ and concavity of the available set *X*. The given task is solved using normal mathematical programming methods.

In practice, it is possible that a situation arises where the set *X* is empty, due to the lack of variables *x* which simultaneously satisfy all the limits, and consequently, the task does not have a solution. In this case, it becomes necessary to refuse to try to solve the input task in a non-fuzzy manner and using fuzzy limits, to formulate the MP task, taking fuzziness into account.

In such cases, due to the impossibility of satisfying all criterial limits simultaneously, it becomes necessary to use compromised schemes for taking into account the requirements of various criterial limits. Compromise ideas and schemes, embedded in direct methods of multi-criterial evaluation of variables, are used for the formulation of FMP tasks and the determination of the solutions to these tasks.

Initially, let reduce the input task to the maximisation of the objective function at points on the *Paretto Set,* of the limits which have been formed:

$$\Pi 1 : \max_{x \in X} \mu_0(x),$$

$$X = \left\{ x : \arg\max \sum_{q=1}^{L} \beta_q \mu_q(x) \wedge \sum_{q=1}^{L} \beta_q = 1 \wedge \beta_q \geq 0, q = 1,2,...,L \right\}$$

The solution of the given task depends on the weighting vector □ and is formed of the control vector (independent variables), values of the objective function and a group of limit values: $x^*(\beta), \mu_0(x^*(\beta)),. \mu_q(x^*(\beta)),..., \mu_L(x(\beta)).$

The following algorithm is proposed in the search for a solution to the task in S1.

Algorithm F1.

1. Let $p_q,\ q = \overline{1,L}$ - the number of steps for each *q*–th coordinate.

2. Determine $h_q = 1/p_q,\ q = \overline{1,L}$ - the size of the steps for the changes to the coordinates of the weighting vector □.

3. Construct the group of weighting vectors $\beta^1, \beta^2, ..., \beta^N$, $N = (p_1 + 1)(p_2 + 1)...(p_L + 1)$ for for variations in the coordinates at intervals of 0.1 with steps h_q.

4. Based on the data obtained from the DM, experts and specialists, determine the term sets for fuzzy parameters and for each limit construct a membership function to satisfy the limits.

5. Solve the task S1 for $\beta^i, i = \overline{1,N}$ and determine the solutions: $x^*(\beta^i), \mu_0(x^*(\beta^i)), \mu_1(x^*(\beta^i), ..., \mu_L(x^*(\beta^i))$.

6. The solutions are presented to the DM for his or her selection of the best ones.

FMP Task 2. Let us consider the situation where it becomes necessary to formulate the FMP task in the presence of several objective functions (criteria), that is MS2.: $\mu_0(x) = (\mu_0^1(x), ..., \mu_0^m(x))$, either the range of priorities *I*={1,2,…,*m*} or the weighting vector reflecting the relative importance of the objective functions (local criteria) $\gamma = (\gamma_1, ..., \gamma_m), \gamma_i \geq 0, i = \overline{1,m}$, $\gamma_1 + .\gamma_2 + ... + \gamma_m = 1$ is known. Then we can come to the following formulation of the multi-criterial FMP task.:

$$\max_{x \in \Omega} \quad \mu_0^i(x), \; i = \overline{2, m}$$

The task, as so formulated, is rarely able to be solved, since it is required that *m* objective functions reach their maximum values at a single point. The universally accepted way out in such a case, is to construct a Paretto Set, from which the DM chooses the best solution:

P2.:
$$\max_{x \in \Omega} \quad \mu_0(x),$$
$$\mu_0(x) = \sum_{i=1}^{m} \gamma_i \mu_0^i(x)$$

In order to solve the multi-criterial fuzzy task P2, we propose the following algorithm.

Algorithm F2.

1. Determine the value of the weighting vector, reflecting the relative importance of local criteria $\gamma = (\gamma_1, ..., \gamma_m)$, $\gamma_i \geq 0, i = 1, m, \gamma_1 + \gamma_2 + ... + \gamma_m = 1.$ based on expert evaluation.

2. If $\mu_0^i(x), i = \overline{1,m}$ and / or □ - is determined fuzzily, then construct term sets and membership functions for them.

3. Solve task P.2:

$$\max_{x \in \Omega} \quad \mu_0(x), \quad \max_{x \in \Omega} \quad \sum_{i=1}^{m} \gamma_i \mu_0^i(x),$$

and for the various values of the weighting vector, determine the group of solutions $x^*(\gamma), \mu_0^1(x^*(\gamma)), \mu_0^m(x^*(\gamma))$.

4. The set of solutions which is obtained is presented to the DM for his or her choice of the best ones.

A more general situation of the formulation of the FMP task with several criteria and several limits, using the examples and principles which have been given, brings together the formulations of the tasks which have already been given. In so doing, we can describe two different approaches.

The first approach involves using for the limits, the available set which has been obtained with various optimisation principles (P.1 etc) and the problem of the formulation of the FMP task with several objective functions is solved using optimisation principles P2 and others, maximising the objective functions for the available set which has been obtained. In the event of fuzzy criteria, their membership functions are maximised.

The second approach involves considering parts of the objective functions as limits and then using for this version the first approach to the formulation of the FMP task.

As an example of the formulation and solution of multi-criterial FMP tasks with several limits (MS 3), let us consider the following task (P3).

FMP Task 3. Let $\mu_0(x) = (\mu_0^1(x),..., \mu_0^m(x))$ be the normalised criteria vector, evaluating the quality of the functioning of the manufacturing facilities. Let us assume that based on expert procedures for each limit $f_q(x),\ q = \overline{1, L}$ a membership function for satisfying the limits is constructed - $\mu_q(x)$. Let either the range of priorities for the local criteria I_k= {1,…,*m*} and limits I_r={1,…,*L*}, or the weighting vector, reflecting the relative importance of the criteria $\gamma = (\gamma_1,...., \gamma_m)$, and limits $\beta = (\beta_1,...., \beta_L)$ be known. Then based on compromised decision making schemes, various multi-criteral FMP tasks can be formulated, with various limits and an algorithm for their solution can be proposed.

For example, based in the *ideas of the main criteria method,* a general FMP task with several criteria and limits, can be given the following formulation of a multi-criterial FMP task:

$$\max_{x\in\Omega}\ \mu_0^i(x), i = \overline{2, m}$$

$$X = \{x : \arg\ \max_{x\in\Omega}\ \mu_q(x), q = \overline{1, L}\}$$

can be written in the following formulation:

P.3.

$$\max_{x\in X} \mu_0^1(x),$$

$$X = \{x : x \in \Omega \wedge \arg(\mu_q(x) \geq \mu_q^r) \wedge \arg(\mu_0^i(x) \geq \mu_r^i), q = \overline{1, L}, i = \overline{2, m}\}$$

The solutions to the given task depend on the limit values - $\mu_1^r,..., \mu_L^r; \mu_r^2,..., \mu_r^m$.

Let us give the structure of the algorithm for solving the multi-criterial FMP task with several limits with the formulation P3.

Algorithm F3.

1. Let us give the range of priorities for the limits $I_r = \{1,....,L\}$ and local criteria $I_k = \{1,....,m\}$ (the main critierium must have priority 1).

2. The DM assigns limit values for the limits $\mu_q^r, q = \overline{1,L}$ and $\mu_r^i, i = \overline{2,m}$.

3. Determine the term sets for fuzzy parameters, based on expert data, and construct the membership functions for satisfying the limits $\mu_q(x) \geq \mu_q^r, q = \overline{1,L}$ и $\mu_9^i(x) \geq \mu_0^i, i = \overline{2,m}$.

4. Solve task P.4. (maximise the main criterium $\mu_0^i(x)$ for the set *X*, taking the limits which have been set into account) and determine the solution:

$$x^*(\mu_r^i, \mu_q^r), \mu_0^1(x^*(\mu_r^i, \mu_q^r)), \mu_0^m(x^*(\mu_r^i, \mu_q^r), \mu_1(x^*(\mu_r^i, \mu_q^r)),..., \mu_L(x^*(\mu_r^i, \mu_q^r)).$$

5. Present the solutions which have been obtained to the DM. If the current results do not satisfy the DM, then he or she assigns new values to μ_r^i, μ_q^r and it becomes necessary to return to step 3, otherwise the search for a solution is concluded and the final results are shown.

In this algorithm, for greater soundness in the assignment by the DM of limit values μ_r^i and μ_q^r, it is necessary to construct dialogue procedures for the assignment of various limit values, analysis of the derived results by the DM and choice of new values of μ_r^i, μ_q^r.

Discussion of Results. Let us consider the results of the research into the development and creation of intelligent systems for decision making for the solution of tasks for ensuring food production safety based on mathematical models and algorithms for multi-criterial optimisation. During the formulation and solution of the given tasks, situations often arise where, for the solution of the task which has been set, in order for efficient decision making, it is necessary to process large volumes of information, consider sets of alternatives, take into account the influence of various factors and evaluate the consequences of one or another decision in conditions of uncertainty. For the solution of such tasks, various assignment systems based on modern information technology have been found to be extremely helpful. Such systems bring together mathematical methods (modelling, optimisation, decision making) and the capabilities of modern computer technology, which makes it possible to significantly improve and accelerate the procedures for the solution of the optimisation task and the finding of effective solutions to the given task.

The main components of intelligent systems which are designed for the effective solution of industrial tasks, including intelligent systems for ensuring the safety of food production, can be treated as the following units: process model package; set of dialogue algorithms for the solution of the set tasks taking into account uncertainty and the fuzziness of input data, knowledge and data bases, a unit for logical inference and explanation of the solution; an identifier of model parameters; a user interface which is linked to the data sources (see Figure 1). Each of these units fulfills a specific function, and their combined functioning allows the user (DM or the person making the decision) to effectively solve the task which has been set [4].

In conditions of uncertainty and with fuzzy input data, it becomes necessary to formalise the knowledge and judgments of the DM, experts and specialists which has a fuzzy nature. In order to solve such fuzzy tasks for decision making, it is necessary to include in the computer systems elements of intelligence, which make possible communication in natural or professional language. These possibilities can be implemented using artificial intelligence methods, including the following elements in the information systems: a knowledge base; a unit for logical inference and explanation of results; algorithms for multi-criterial fuzzy optimisation; and an intelligent interface.f

Intelligent systems with the above listed components are multi-functional. The main functions of such systems include:

- system modeling of various working regimes of the system being researched (for industrial food production) in dialogue with the user, where the results of the modelling are presented in a clear and convenient form;

- support of the decision making tasks for the control of objects in the food production industry;

- processing of recommendations for the adjustment of the object's regime parameters with the aim of obtaining the required values of the critieria in order to ensure the safety and quality of food production;

- processing and the effective presentation and storage of the necessary information (shift logs, reports etc) in the data and knowledge base;

- the prognosis and evaluation of unmeasurable values and indicators which ensure the quality of the functioning of the system, and ensure the safety of production;

- the operational review of the current values of regime parameters of the object for industrial food production;

- the detection and warning of emergency conditions, the issue of detailed recommendations in order to eliminate them, and the training of industrial personnel.

Let us consider the description of the main functional units of the intelligent system which has been created for ensuring the safety of food production.

The *model package unit* contains various models, including fuzzy models, for the individual elements of the industrial system for food production, which have been combined into a single package, allowing system modelling of the functioning of the system to be carried out as a whole. These models are designed to determine (calculate) the values of local criteria (indicators of the quality of the functioning of the production) depending on the values of input signals (control, process regime parameters, industrial situation indicators). The development of process models for industrial systems can be carried out using a variety of approaches. For example, based on the decomposition principle, initially models of the separate sub-systems (assemblies, installations, divisions) of the industrial system are constructed. In so doing, depending on the data which has been collected (a priori knowledge, statistical data, qualitative (fuzzy) data), various models may be constructed (determinate, stochastic, fuzzy), which must then be combined into a system (package) in accordance with the material, energy, data and other flows which are present.

Set of Dialogue Algorithms: ie *A*lgorithms for fuzzy mathematical programming and multi-criterial fuzzy optimisation designed for the solution of tasks for the ensuring of the safety of food production, taking into account the fuzzy nature of input data. These algorithms, based on a package of models, knowledge bases and a logical inference block implement the search for rational working regimes of the object according to the criteria which have been chosen, and determine the recommended values for control inputs which provide for such regimes. The choice of the final solution, as a rule, is made by the DM.

The logical inference and explanation of results unit is formed by the implementation of the inference method, prompts and the explanation of the results which have been obtained. This unit, based on the algorithms for the solution of the tasks which have been set and the knowledge and data bases, provides the DM (industrial personnel, operator) with recommendations in the process of operational control of production and forms control inputs. These procedures are carried out by means of the creation of fuzzy sets and the carrying out of operations on them based on logical (compositional) inference rules.

Recommendations, presented to the DM, may, if desired, be accompanied by explanations, giving a justification for the flow of the inference process. System users often desire to be convinced that the conclusions and recommendations provided to them by the system are justified. Explanation of the results which have been derived, in a form which is compact and convenient for human analysis, is implemented by means of the fixation of all arguments which have been used for alternative choices. For the formulation of the explanation, the unit being described is linked to the algorithms for multi-criterial selection and the knowledge base.

The knowledge and data base is designed for the storage of the formalised knowledge of experts and specialists or researchers in the field under consideration and statistical data concerning the production. Data from this unit is used in the process of the analysis and selection of solutions, for the explanation of selection results, for the drawing up of industrial reports and for the adaption of models to new conditions.

In practice, several methods for the presentation of knowledge in intelligent systems are known [1, 23]: logical (predictive calculus): logical-linguistic (predictive calculus based on fuzzy set theory); relational (tabulated); network graph-grammar based; frame-based (structured framework) and production. The most convenient form for the formulation and presentation of knowledge in the IDMS (Intelligent Decision Making System) for the control of production in conditions of uncertainty, is fuzzy outputs, having the construction [2, 24]:

IF $\widetilde{x}_i \in \widetilde{A}_i$, *THEN* $\widetilde{y}_j \in \widetilde{B}_j$,

where $\widetilde{x}_i,\ i = \overline{1,n}$, $\widetilde{y}_j,\ j = \overline{1,m}$ are accordingly the input and output linguistic variables of the object; $\widetilde{A}_i \subseteq X_i, \widetilde{B}_j \subseteq Y_j$ - are the fuzzy sub-sets, characterising the corresponding input and output parameters of the object.

Every such rule is charcterised by one or more equivalent states of the object, as established by experts and specialists, the control of which, taking into account the actual conditions, will be sufficiently close to optimal. Information concerning actual industrial situations of the object for the selection of various values of the input linguistic variables, and various rules for the functioning of the object etc, are stored in the knowledge base.

Production rules are graph-grammar based models for the representation of procedural knowledge concerning the industrial object, which is formally described in the form [25]:

IF (situation) *THEN* (action);

IF (reason), *THEN* (consequence) etc

The application of production rules allows a system to be created based on rules which have the following advantages:

- a quick response to changes within wide limits and to many unforeseen situations in the external environment, is ensured;

- individual production rules may be independently added to the knowledge base, or excluded from it, or adjusted;

- uniformity is achieved in the presentation of knowledge in the knowledge base, which makes it easier for human operators to understand or easier for other sub-systems to interpret.

Disadvantages in the use of the production method of the representation of knowledge as a style of programming include the fact that it makes it more difficult to understand and verify the programme, and the computational costs and resources required are multiplied several times over. In order to solves these problems, it is necessary to use a broad system of prompts and commentaries in the programmes and make use of the possibilities of parallel computations.

In order to ensure mutual understanding between the user and the system unit, the knowledge and data bases must include two sorts of knowledge: knowledge of the professional language of the user and knowledge of the particular problem area for which the IDMS has been created.

The Identifier of Parameters is itself a programme which carries out the verification of the adequacy of the model in the given conditions and the working regime of the object and, if necessary, executes recalculation (identification) or the model parameters. In order to do so, the identifier uses data concerning the current values of regime parameters, as well as data from the knowledge and data bases. In the event of changes to the state of the object and the conditions of the processes, the unit adjusts the model of the actual object and the current situation of production and may use the differential values of the computations (by model) and the experimental data (according to production indicators), for example:

$$R = \sum_{j=1}^{m} \left| y_j^M - y_j^Э \right| \le R_{доп}$$

where y_j^M , $y_j^Э$ - are accordingly the modelled and experimental values of the indicators; R_{adm} – is the admissible deviation (accuracy of the model).

We note that the experimental data may be obtained based on expert evaluation and may have a qualitative nature. In this case $\widetilde{R}$ is fuzzy in nature, and in order to calculate it, the methodology of fuzzy set theory is used. Control inputs should be selected in such a manner so as to minimise the value of criterium *R.*

The User Interface is designed to provide a convenient dialogue regime of working between the user and the system, in the solution of the given task, and also in the implementation of a range of other functions of the IDMS. During the process of working with the system, as is necessary, the following are implemented: output in the display of a diagramme of the production facilities (production flow chart) and data concerning the state of the facilities; display on the screen of the values of control parameters and the computational results obtained in a form which is convenient for the user (tables, graphs, diagrammes, text); visual observation of the process of optimisation of the working regime of the facilities and the ensuring of the safety of food production; input and adjustment of the necessary parameters for the control of production etc.

The dialogue should be carried out using the professionally oriented language of the user. Professionally oriented language is a small sub-set of natural language and has a range of properties which facilitate the mutual understanding of specialists of the essence of the problem being discussed in the facilities being researched. There should be no synonyms in such language, that is, each word should be unambiguous. Phrases and text in professionally oriented language are constructed using stricter rules than phrases and texts in natural language. However this 'light' language is still much more convenient and understandable for the user of the IDMS, that is the DM or industrial staff.

In order for the system to be able to understand professionally oriented language and the formulation of the task which the user wants to communicate to the system, it must have corresponding units: dialogue control algorithms, a package of models of the object, and a knowledge base, which we have considered above. The level of intelligence of the interface depends on the simplicity and convenience of the communication between the user and the system and on the volume and contents of the information stored in the knowledge base concerning professionally oriented language in the problem area.

The effectiveness of the intelligent system which has been developed for the solution of the task of ensuring the safety of food production is determined by the quality of the way that knowledge is formulated and presented, by the models developed, by the algorithms for solving the task, as well as by the user-friendliness of the interface.

In the years to come, the communication between the user and the computer should reach a new level of quality. Instead of communication via text and using a keyboard, in the future there may be verbal communication, where the user inputs the required information by voice and receives messages from the system in the same form. The verbal form, which is the habitual means of human communication, makes communication with the intelligent system more comfortable and greatly increases the effectiveness of such systems.

In contrast to other known methods, the methods which are proposed in this paper for the formulation and solution of FMP tasks, preserve the fuzziness of input data which has been obtained in the form of descriptions of criteria and limits, and based on various compromise decision making schemes, in a form which is convenient for the DM, the problem of multiple criteria is resolved. This makes it possible to more adequately describe the manufacturing situation in a fuzzy environment and obtain effective solutions of the tasks which arise for the control of complex manufacturing facilities which are difficult to describe quantitatively.

Conclusion:

An approach to the creation of intelligent systems to be used for the solution of tasks in the ensuring of the safety of food production has been proposed. The legal basis and international standards for the ensuring of the safety of food production have been studied and analysed. The methods and resources for the creation of expert systems for ensuring the safety of food production have been considered. The main steps for the creation of intelligent systems for the solution of the tasks of the ensuring of the safety of food production have been proposed and described. The architecture of an intelligent system for decision making for the ensuring of the safety of food production has been constructed, and the main functional units have been described. The resources required to implement the intelligent system for ensuring the safety of food production have been considered.

Several manufacturing situations in the food production industry have been singled out, for which the tasks to be solved have been formulated, with fuzzy input data, in the form of fuzzy mathematical programming tasks, and heuristic algorithms for their solution have been developed. The originality of the work lies in the fact that, in contrast to other known methods, the proposed methods for the formulation and solution of the FMP tasks preserve the fuzziness of input data which has been obtained in the process of the description of criteria and limits, and based on various compromise decision making schemes, in a form which is convenient for the DM, the problem of multi-criteriality is resolved. This makes it possible to more adequately describe the manufacturing situations in a fuzzy environment and achieve the effective resolution of the tasks which have been formulated for the ensuring of the safety of food production in conditions of multi-criteriality and fuzzy input data.

This approach to intelligent systems to support decision making in the solution of the tasks for the ensuring of the safety of food production, is being proposed for the first time in this particular sphere, based on a systematic approach, the development of models for the task to be solved, and using the technology of expert systems and fuzzy set theory methods.

According to the results of the research which has been carried out, the following recommendations can be made aimed at raising the level of safety of food production:

- Food production safety strategies should be based on an assessment of risk, leading to a range of priorities for measures which can give results in the form of the greatest reduction in the rate of prevalence of food borne diseases;

- It is necessary to take a complex multi-disciplinary approach to the safety of food products, which encompasses the whole production chain, and the processing and distribution of food products. This implies an increased level of control on animal feed and other aspects of primary production;

- Persons involved in the production, processing or distribution of food products, must be subject to internal system controls, based on an HACCP approach;

- In order to ensure the effective scientifically based solutions to the tasks of the ensuring of food production safety, it is necessary to create and implement intelligent systems based on artificial intelligence methods, fuzzy set theory and modern computer systems.

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Keywords: intelligent systems, food production safety, Paretto optimisation, multi-criterial optimisation

[P.023]

A predictive modelling study for using high hydrostatic pressure, a food processing technology, for protein extraction

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The aim of this study is to fit a response model to one response, extracted protein concentration by using high hydrostatic pressure, a food processing technology, as a function of two particular controllable factors of extraction procedure. These factors are “pressure” (applied in MPa) and the “extraction solvent”. Data were taken from a previously published data [1], where the minimum and maximum values chosen for pressure were 100 MPa and 300 MPa with a center point of 200 MPa. The solvents were PBS, TCA and Tris-HCl. Protein concentration values were the mean values of 3 replicates.

Firstly, a regression statistics were conducted by the data mentioned above to identify coefficients for intercept, pressure and solvents. The coefficients for intercept, pressure and solvents were identified as 34.29753333, 0.008442 and 0.85425 respectively with *p*-values of 0.03 for pressure and 0.10 for solvents.

A predictive analysis model was fitted to the protein concentration response by using the predictive analysis model proposed with the analysis conducted.

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Keywords: Predictive modelling, High hydrostatic pressure, Food processing technology, Protein extraction

[P.024]

Predicting the microbial shelf-life of naturally contaminated packaged cooked meat products: Possibilities and limitations

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This study aims at assessing the performance of existing models in predicting microbial shelf life of packaged cooked meat products. Most kinetic growth models focus on growth of pathogens and their relevance to industry has already been proven. Kinetic models that predict shelf-life, however, are scarce and mostly not based on naturally contaminated products. Therefore, their relevance to industrial applications is still unknown.

In cooperation with 11 Belgian cooked meat producers, an extensive analysis of industrial products (cooked ham, pâté, sausages,…) was performed on the evolution of microbial counts (total aerobic count [TAC], lactic acid bacteria [LAB] and *B. thermosphacta*), pH, gas composition, dry matter, a_w, lactic acid, acetic acid and salt content. A selection of isolates representing specific spoilage organisms were identified through DNA fingerprinting (Amplified Fragment Length Polymorphism [AFLP] analysis) or sequencing of 16S rRNA or *phe*S genes. For each product, several individual packages were sampled on the same day to quantify the natural variability in microbial proliferation.

Results showed large variations in initial contamination levels (< 0 – 4.3 log CFU/g; average 1.7 log CFU/g), independent of product or production plant. Due to low initial contamination levels and because individual commercial packages were analysed, a high variability in microbial count was observed during the lag phase and exponential growth phase. Hence, it was in some cases impossible to fit a growth curve to the data. In approximately 70% of the naturally contaminated products, a clear lag phase was observed which cannot be predicted by the existing kinetic models. The microbiota at the end of the storage period was very diverse, including several LAB groups (*Lactobacillus* sp., *Leuconostoc* sp., *Carnobacterium* sp.,…), but in various products the microbial count was dominated by non-LAB.

This case study on cooked meat products illustrates the limitations of kinetic models to predict the actual microbial shelf-life of food products.

Keywords: cooked meat, naturally contaminated, specific spoilage organisms, industrial practice

[P.025]

Final destination: Outcome of heat treated geobacillus stearothermophilus spores during storage

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Geobacillus stearothermophilus is a well-known flat sour spoilage organism in canned food. The heat resistance of its spores was widely investigated, however the outcome of heat treated surviving spores during storage of canned food remains unexplored.

The purpose of this work is to study and to model the behavior of the surviving heat treated spores during storage using both classical plate counting and flow cytometry method. The model should take in account the heat treatment conditions, and storage conditions (temperature, pH).

Spores of *G. stearothermophilus* was heat treated at four different intensity, and stored in nutrient broth or in phosphate buffer at conditions not allowing growth The spore behaviour was evaluated by count plating and by flow cytometry with Syto9 staining.

After a heat treatment, the surviving spores able to recover on agar medium decreased during the storage in no-growth conditions. Inactivation kinetics during storage are biphasic, suggesting the presence of two subpopulations. The resistances of both sensitive and resistant subpopulations to storage stress were affected by the pH and the temperature of storage. Count plate results revealed that neutral pH and refrigerated temperature give a higher resistance to storage stress for the surviving heat treated spores. The flow cytometry results showed that those heat treated spores undergo germination under no-growth storage conditions. And the loss of viability is related to the transition of spores from the refractive to the permeabilized state (dark phase). The rate and the yield of permeabilization were modeled. Like in the case of plate count method, the values of these two parameters were dependant of heat treatment and storage conditions.

This work provides a new perspective to deal with flat sour spoilage by taking in account the heat treatment efficiency and the impact of the physiochemical food properties during the storage.

Keywords: Geobacillus stearothermophilus, Storage, Modeling, Flow cytometry

[P.026]

Modelling the probability of growth and aflatoxin B1 production of Aspergillus flavus under changing temperature conditions in pistachio nuts

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The aim of this work was to use probability models for the prediction of growth and aflatoxin production by *Aspergillus flavus* as a strategy to mitigate the aflatoxin presence in pistachio nuts during postharvest. Human exposure to aflatoxins in foods is of great concern, then the development of models for prediction of growth of aflatoxigenic fungi becomes a key step in risk management.

A.flavus was isolated from pistachio nuts and its probability of growth and aflatoxin B_1 (AFB1) production, under static and fluctuating conditions were studied. A full factorial design with five constant temperature levels and four changing temperature regimes (between 15 and 25 ºC) was used. A cocktail inoculum of 25 *A. flavus* isolates was used and water activity was adjusted to 0.87. Logistic models, with temperature and time as explanatory variables, were fitted to the probability of growth and AFB1 production under a constant temperature level, afterwards they were used to predict probabilities under non-isothermal scenarios.

The models obtained showed levels of concordance from 80 to 100% in most of the cases. Moreover, the presence of AFB1 in pistachio nuts could be correctly predicted through AFB1 models developed in agar medium or through growth models in pistachio nuts. These findings can support decision making, at transport and storage level, and could be used by producers and processors to predict the time for AFB1 production by *A. flavus* in pistachio nuts in postharvest.

Keywords: Aspergillus flavus, aflatoxin, pistachio, non-isothermal

[P.027]

Reaction-diffusion modelling of oxygen dynamics in fresh meat

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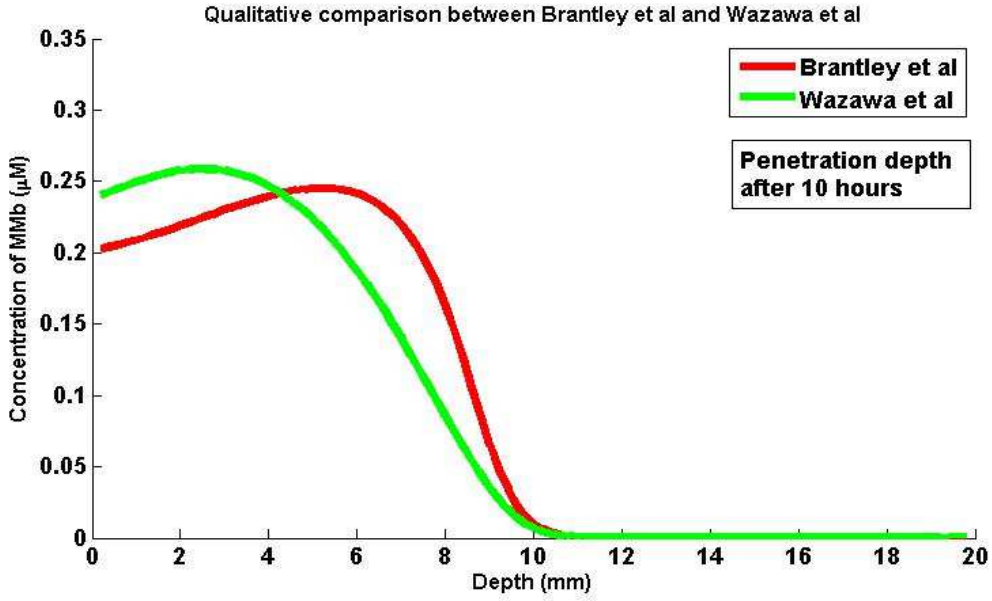
Fresh meat is packed and stored prior to consumption using modified atmospheric packaging (MAP), in-which the meat is packed in an atmosphere of mostly O_2. The main function of oxygen is to preserve the red color preferred by consumers. The red color is a result of oxygen reacting with the tissue myoglobin (Mb) to make oxymyoglobin (MbO_2). Further oxidation leads to change in Mb and MbO_2 producing metmyoglobin (MMb). In this state the meat becomes brown which is not attractive to consumers. Therefore it is useful to have a mathematical model that could predict the optimal MAP.

We compare the two different models by Wazawa *et al*. [Biophys. J. 63, 544-550, (1992)] and Brantley *et al*.[J. Biol. Chem, 268, 6995, (1993)] adding an oxygen-diffusion term. The models are compared to how well they fit real data, across different temperatures, and times. It is also examined if one can be fit to the other, and preliminary results suggest that the 2 different models are in fact qualitatively different.

It is also found that the dynamics of oxygen is primarily diffusion controlled.

This is a new approach to make correct physical models rather than statistical models in the prediction of fresh meat shelf-life.



A qualitative comparison between Wazawa *et al*. and Brantley *et al*. after 10 hours where Brantley model has been fitted to the Wazawa model; notice how the MMb peak, associated with the brown color appear under the surface.

Keywords: Diffusion, Meat, Oxygen, Myoglobin

[P.028]

Characterising the microbiological safety of Linguiça, a Portuguese traditional dry-fermented sausage

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Linguiça is a Portuguese traditional sausage whereby diced meat is macerated in water, wine, salt, garlic and pepper, followed by stuffing, maturation, smoking and drying at low temperatures. Since these sausages are typically produced in small regional processing units, their microbiological safety can be variable. Thus, the objective of this study was to investigate the growth of hygiene indicators (total viable counts and *Enterobacteriaceae*) and selected pathogens (*Staphylococcus aureus* and *Listeria monocytogenes*) during processing of *Linguiça*. Samples were taken from a total of six batches from two regional factories. For each batch, sampling visits were conducted to determine microbial counts in casings, ingredients, raw meat, batter right after mixing with ingredients and at the end of maceration, sausages after smoking and after drying, as well as contamination from raw materials and ingredients. A mixed-effects model was fitted for each bacterial group in order to assess the effects of factory, time nested in stage (mixing with ingredients, macerating, smoking and drying) and casings. On a batch basis, higher counts of TVC and *S. aureus* in casings were positively associated with significant increase of these bacterial groups in meat after stuffing and smoking. *Enterobacteriaceae* counts decreased significantly during ripening up to levels below 3 log CFU/g, which can be due to the proliferation of fermentative microflora, smoking and a decrease in Aw. Contrarily*, S. aureus* increased their numbers significantly during mixing and after stuffing, implying that operators and ingredients are important sources of contamination. *L. monocytogenes* entered the product mainly through raw meat, yet a decrease in occurrence was demonstrated during ripening. Results showed that neither fermentation metabolites nor final product characteristics ensures sufficient inhibition of these pathogens. There is a need to develop strategies to control *L. monocytogenes* as well as to reinforce good hygiene and manufacture practices among regional producers.

Keywords: sausage processing, Staphylococcus aureus, Listeria monocytogenes, dry-curing

[P.029]

Prediction of potential bioactive peptides from Red Algae species (Gelidium sp, Palmaria palmata and Porphyra sp) using bioinformatics tools

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Macroalgae have been traditionally consumed in Asia and commercially used in the world as sources for carrageenan, agar (red algae), fucoidan, laminarin (brown algae), pharmaceutical and biomass production. Recently, macroalgae are regarded as great potential sources of plant proteins with increasing need. Among macroalgae groups, red algae contain relatively high protein content ranging from 2.7 to 47.0% (dry basis) compared to green and brown algae. Some studies have indicated that algae proteins can be abundant precursors for producing bioactive peptides, although the relevant research on structural information is fairly limited. The research objectives were to predict bioactive peptides and to investigate the potential biological activities in three red algae species (*Gelidium sp, Palmaria palmata* and *Porphyra sp*) using bioinformatics tools (UniProtKB and BIOPEP database). Protein sequences of *Gelidium sp, Palmaria palmata* and *Porphyra sp* were obtained from the UniProtKB database; the subsequent analysis was carried out using BIOPEP database to predict bioactive peptides with reported biological activities. Experimental results revealed that three species of red algae showed high occurrence frequencies (0.350-0.433) in angiotensin-converting enzyme-inhibitor activity with bioactive peptide sequences such as RL, AG, DA, GD, PG and EA. Several potent biological activities such as dipeptidyl peptidase inhibitor (LA, GP, VA, FA, LP), antioxidative (AY, YSY, LK, VY) antiamnestic (PG) and antithrombotic (PG) activities were also observed; in addition, anticancer activity (VVV) can be found in *Palmaria palmata* species. Predictive results evidence that this approach is effective to evaluate and predict bioactive peptides of algae proteins.

Keywords: Bioactive peptides, Red algae, Bioinformatics tools

[P.030]

Modelling the growth of Salmonella spp. and Escherichia coli O157 on lettuce

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Salmonella spp. and *Escherichia coli* O157 are important food pathogens worldwide and in several foodborne outbreaks lettuces have been identified as the food vehicle of these microorganisms. Based on these facts, this study is aimed to model the growth prediction of *Salmonella* spp. and *E. coli* O157 on lettuces exposed to different temperatures.

Salmonella spp. and *E. coli* O157 were inoculated separately on lettuce and stored at 5 (*Salmonella* spp. only), 10, 25 and 37°C. Growth curves were built by fitting the data to the Baranyi's DMFit model. Secondary models were fitted with Ratkowsky equation.

Experimental data showed that both *Salmonella* spp. and *E. coli* O157 grow at every temperatures examined. The lag phase for *Salmonella* spp. at 5°C was 60 hours. At 10°C, the lag phase for *Salmonella* spp. and *E. coli* O157 were respectively 24 and 50 hours. At 25°C, the lag phase for *Salmonella* spp. and *E. coli* O157 were respectively 2 and 3 hours. At 37°C, the lag phase for *Salmonella* spp. and *E. coli* O157 were respectively 1 and 2 hours. The maximum growth rate for *Salmonella* spp. at 5°C was 0.02 log CFU/h. At 10°C, the maximum growth rate for *Salmonella* spp. and *E. coli* O157 were respectively 0.05 and 0.02 log CFU/h. At 25°C, the maximum growth rate for *Salmonella* spp. and *E. coli* O157 were respectively 0.63 and 0.71 log CFU/h. At 37°C, the maximum growth rate for *Salmonella* spp. and *E. coli* O157 were respectively 0.82 and 0.79 log CFU/h.

In conclusion, the developed models could be used to assess the growth of both *Salmonella* spp. and *E. coli* O157 on lettuce under various temperatures, ranging from 5 to 37°C and 10 to 37°C, respectively.

Keywords: lettuce, modelling, Salmonella spp., Escherichia coli O157

[P.031]

Mechanistically modeling the transition periods between lag / exponential and exponential / stationary phases of Escherichia coli K-12

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A continuing goal in predictive microbiology is models based on physiological behavior. Buchanan et al. (1997) hypothesized that (1) the curvilinear lag/exponential transition represents the variability of cells in the adjustment (t_a) and metabolic (t_m) periods, and (2) the exponential/stationary transition is determined by limiting nutrient diffusion rates. The current studies provide data to test hypotheses.

Nutritional-shift trials were conducted to quantify the lag phase of *E.coli* K-12. *E.coli* K-12 cells were cultured in tryptic soy broth without dextrose (TSB-G) and then transferred to TSB-G and TSB with lactose (TSB+L) and cultured at 15-40^{o}C. Growth was measured spectrophotometrically and culturally. Lactase activity was assayed by ONPG method. The exponential / stationary phase transition was studied by culturing *E. coli* at different agitation rates and incubation temperatures using similar procedures. Data were fitted using the two- or three-phase linear model (IPMP 2013).

The spectrophotometric and viable count data fit the primary growth models well and displayed a similar temperature tendency, and provided estimates of t_a and t_m. Shifts from a carbohydrate-free medium to a lactose-containing medium resulted in an induction of lactase activity. The time of lactase production suggest that the translation of lactase gene occurs after completion of lag phase. Agitation rates influenced the shape of the exponential/stationary phase transition, with higher rates resulting in a more abrupt transition. Using data from these trials in Monte Carlo simulations allowed the generation of traditional sigmoidal growth curves while simultaneously allowing examination of phase-dependent physiological events.

Keywords: transition period, lag phase, three-phase linear model

[P.032]

The mode of action of weak organic acids in Saccharomyces cerevisiae; A network approach

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Weak-acid preservatives are well known to inhibit growth of microorganisms in foods and beverages. They appear to share a common mode of action, despite their various chemical structures. They are more effective at low pH values where solutions contain increased concentrations of associated acids. These associated molecules can pass the plasma membrane followed by dissociation of these molecules, resulting in acidification of the cytosol. Ullah 2012, presented kinetic data on the effect of weak acids on cytosolic pH of Saccharomyces cerevisiae. An apparent difference on the rate of acidification was found for acetic acid on the one side and sorbic and benzoic acids on the other side. Propionic acid was in between. Based on these data we developed a secondary Gompertz model that describes the intracellular acidification rate in. In the model associated organic acid and the 'intrinsic' lipophilic properties of the acid are incorporated in the model. Besides we are currently developing a more mechanistic model that uses the above mentioned factors but in this model the buffer capacity of the yeast cell was incorporated. The main factor is probably not the proteins but phosphate buffer (estimated as 40 mMol). By taking into account the buffer capacity the total influx of protons could be estimated. The majority of proton influx is not reflected by the intracellular pH but the protons absorbed by the buffer. By using the above mentioned factors and the reaction constants underlying the Ka values of the organic acids a set of differential equations was formulated that describe the association and dissociation of the acids outside the cell, over the cell membrane and inside the cell. Finally the rate of diffusion inside the cell will be taken into account. Thus the effect of organic and inorganic acids on the growth of yeasts may be predicted.

Keywords: Intracellular, adcidification, mechanistic, influx

[P.033]

Modeling the effect of dioxygen concentration on growth of aerobic, anaerobic and aero-anaerobic bacteria

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Combined with a modified atmosphere, packaging helps to increase the food shelf life and to improve food safety. Eventhough modified atmospheres are generally composed of carbon dioxide and nitrogen, residual oxygen may remain in industrial packing.

This work aims at studying and modeling the impact of dioxygen concentrations on microbial growth and assess whether residual oxygen concentrations allow aerobic microflora development or inhibit anaerobic microflora.

Pseudomonas fluorescens and *Listeria monocytogenes* were cultured in BHI agar medium whereas *Clostridium perfringens* was cultured in RCM. All petri-dishes were prepared in advance, stored in an hypoxia laminar flow hood, then inoculated and incubated at 25 °C. Dioxygen concentrations ranged from 0.1% to 6% for *Clostridium* and from 0.1% to 20.9% for the two other strains. To determine kinetics, 15 sampling were performed for given conditions of atmospheric O2. Growth rate was estimated by mathematical fitting.

With only 0.1% O2, growth of *Pseudomonas* was observed and increased with the concentrations till up to 5% O_2 where it reached a plateau. For *Clostridium perfringens*, oxygen acts as an inhibitor until 6% where bacteria stop growing. For *Listeria monocytogenes*, there was no influence of the O_2 levels on the growth, however experimental data suggested an impact on the lag time.

The ANR MAP'OPT project main deliverable consists in a modeling tool addressed to food industrials to account for the impact of both O_2 and CO_2 on bacterial growth and gas transfer through packaging. In the presence of oxygen, the growth of aerobic bacteria can modify the initial composition of the gaseous atmosphere in the head space and in the food and can influence its efficiency These promising results underline the possibility to implement new factors in predictive modeling tools, optimize packaging size and composition, further reduce volume, guarantee shelf life without preservative and reduce waste.

Keywords: residual oxygen, Listeria monocytogenes, Pseudomonas fluorescens, Clostridium

[P.034]

Computer simulation analyses to improve radio frequency (RF) heating uniformity in dried fruits for insect control

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In recent years, radio frequency (RF) heating has been investigated as a rapid and safe postharvest disinfestation method for agricultural commodities. However, major challenges in adopting RF heating in the food industry are non-uniform heating and, sometimes, runaway heating. In this study, An experimentally validated computer simulation model was used to investigate effects of package geometries, electrode configurations, and forced air at 60 °C on the RF heating uniformity of raisins for insect control. Higher temperatures (60–76 °C) were observed in the areas between the edges and corners in the top and bottom layers of the RF treated raisins in rectangular and cylinder shaped containers, while lower temperatures (40–52 °C) were observed at the central areas of each layer of the RF treated raisins. The heating uniformity was improved by rounding corners and reducing edges of the package geometry, modifying electrode configurations, and applying heated forced air after RF heating. About 356 min were needed to raise the raisin temperature in a rectangular geometry (25.5 x 15.0 x 10.0 cm^3) from 23 ºC to 55–60 ºC using only forced air treatment at 60 ºC. In contrast, only 6 min of RF heating followed by 10 min of forced air at 60 ºC were needed to reach a sample temperature of 55–60 ºC when using electrodes with an area smaller by 2 cm than the treated sample.. This technique to improve the uniformity of RF heating can be implemented to develop a postharvest treatment protocol to control insects in packaged dried fruits.

Keywords: Computer simulation, Dried fruits, Heating uniformity, RF heating

[P.035]

Analysis of vacuum packed beef regarding psychrotrophic bacteria growth and biogenic amines content

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It has been recognized that biogenic amines content in meat can be considered as a freshness marker. Considerable amounts of some biogenic amines can appear during food storage under certain conditions, according to the handling of the raw material, technology applied, storage temperature and time, packaging condition, mainly if amino acid - decarboxylase positive microorganisms are present. The aim of this study was to evaluate the microbial growth and metabolic production of biogenic amines during chill storage of beef. The vacuum packed beef cuts (*Longissimus dorsi* muscle) were analyzed over storage at 0, 15, 30, 45, and 60 days at 7°C to determine the psychrotrophic bacteria growth and the biogenic amines amount. The biogenic amines considered were: putrescine, cadaverine, histamine, spermidine, and spermine. The biogenic amines quantitative determination was carried out by means of reversed phase high performance liquid chromatography (HPLC) with UV detection. The analyses were performed in nine samples. Statistic procedures were performed using SAS statistical software. The growth parameters including lag time (LT), maximum specific growth rate (μ_{max}), maximum bacterial cell density (y_{max}) and coefficient of determination (R^2) in the primary model were determined according to Baranyi model. The values of histamine and spermidine increased significantly ($P < 0.001$) during storage time, while the levels of spermine decreased ($P < 0.001$). Cadaverine and putrescine showed a high standard deviation after 15 days of storage. Psychrotrophic bacteria counts increased significantly ($P < 0.001$) reaching 7.6 log cuf/g over time. Psychrotrophic bacteria counts positively correlated to histamine and spermidine ($r = 0.68$ and 0.61, respectively), while with spermine there was a negative correlation ($r = -0.70$). Conversely, no significant correlation was found between psychrotrophics counts, putrescine and cadaverine.

Keywords: biogenic amine, biodeterioration, vacuum packed beef, freshness markers

[P.036]

16S rRNA gene sequencing as a tool to study microbial populations in foods and process environments - Limitations and opportunities

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Methodological constraints during culturing and biochemical testing have left the true microbiological diversity of foods and process environments unexplored. Culture-independent molecular methods, such as 16S rRNA gene sequencing, may provide deeper insight into microbial communities and their role in food safety by e.g. supplying novel data for predictive food modeling as has previously been suggested. During method optimization, we have identified several factors, which distort the characterization of the population, including PCR inhibitors present in samples, DNA extraction methods, choice of primers, DNA polymerases, databases used for taxonomic assignments, and most importantly the fragment of the 16S rRNA gene which is analyzed. This study investigated microbial communities in meat and the meat process environment with special focus on the *Enterobacteriaceae* family as a subpopulation comprising enteropathogens including *Salmonella*. Samples were analyzed by a nested PCR approach combined with Illumina®16S DNA sequencing and standardized culture methods as cross reference. We have shown that the V4 variable region of the 16S rRNA gene was not suitable to differentiate between members of the *Enterobacteriaceae* family; however a better separation between genera was achieved by sequencing both the V3 and V4 variable region. Taxonomic assignments of sequences were especially affected by the variable region and the DNA extraction method, but less by the polymerase. Different DNA extraction methods affected both alpha and beta diversity. However, relative abundances and taxonomic assignments for each sample using the same method were very reproducible, which allows for quantitative estimations of subpopulations using culturing of specific bacterial groups as anchor points. Altogether, we have shown that conclusions from population studies based on 16S rRNA gene sequencing need to be made with caution. Overcoming the constraints, we believe that population studies can give new research possibilities for e.g. interaction studies, identification and growth of indicator organisms, or source attribution.

Keywords: 16S sequencing, meat, Enterobacteriaceae, population studies

[P.037]

Recording of latent infections as a model to forecast of brown rot

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Brown rot is one of the most important diseases of stone trees worldwide. This disease causes shoot blight, pre- and post-harvest fruit rots. The management of brown rot is very difficult especially in wet areas. Plant disease forecasting systems have been developed to help growers make economic decisions about disease management. The aim of this study was to evaluate a disease forecast model to predict brown rot, caused from the fungi of genus *Monilinia*. The existence of latent infections in different peach – nectarine cultivars were investigated using the ONFIT methods and correlated with the percentage of pre- and postharvest fruit rots. The results showed that the percentage of latent infections was differed among the peach – nectarine cultivars tested. There was no correlation between the percentage of latent infections and the pre-harvest fruit rots. However, the cultivars with the higher percentage of latent infection showed higher percentage of postharvest fruit rots.

Based on the above results, it can be concluded that the management of the latent infections during the vegetative period could be regarded as an efficient method to reduce the percentage of postharvest fruit rots

Keywords: Monilinia, Fruit rot, Peach trees

[P.038]

Modelling dose-response curves of Escherichia coli ATCC 35218 exposed to hydrogen peroxide with the Fermi distribution function

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Hydrogen peroxide (H_2O_2) has been proposed for decontaminating fruit and vegetables due to its low toxicity and safe decomposition products. This study was aimed to model the dose-response curves of planktonic cells of *E.coli* ATCC 35218 exposed to H_2O_2 at different temperatures, pH's and sanitizer concentrations using the Fermi distribution.

Triplicate inactivation experiments of stationary phase *E. coli* cells (3 - 5 x 10^7 CFU/mL) were carried out at 12.5, 25.0, 37.5 and 50.0°C in 250 mL Erlenmeyer flasks with 99mL of sterile citric acid-Na_2HPO_4 buffer solutions (pH 3.0; 5.8 and 7.2) containing different H_2O_2 concentrations (0 to 6.00 %w/v). Aliquots (1000 µL) were periodically collected, neutralized with buffered (pH 7.0) 4% w/v $Na_2S_2O_3.5H_2O$ solution to stop the inactivation reaction, serially diluted in 0.1% peptone water and pour plated in duplicate using PCA. The plates were counted following incubation (37°C, 48 h). Dose-response curves were represented as the surviving fraction (S(X)) versus the dose of the lethal agent (X). The Fermi distribution was used to describe the spectrum of resistances of the population to H_2O_2 treatment, with the corresponding parameters k (which represents the distribution' spread) and Xc (the mode and mean of the lethal agent intensity at which organisms are destroyed).

Dose-response curves pattern depended on temperature and pH. Despite the symmetric assumption around the mean, Fermi equation goodness of fit was adequate (R^2_{adj} 0.96-0.99). Residual plots were generated and the normality of residuals distribution was verified. Xc and k parameters significantly decreased as temperature increased and pH decreased. For instance, at 25.0ºC, for short exposure times and high sanitizer concentrations, Xc value was doubled when the pH varied from 3.0 to 7.2.

The model quantified the effects of pH and temperature in terms of two parameters with intuitively clear meaning and gave useful information regarding the sanitation process.

Keywords: E. coli, hydrogen peroxide, dose-response curves, Fermi distribution

[P.039]

Modeling the enhanced thermal inactivation of Cronobacter sakazakiiby inclusion of "parabens"

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The ultimate goal of this study is to acquire the data needed to develop mathematical models that describe the enhanced thermal inactivation of *Cronobacter sakazakii* by the inclusion of "parabens." The key parameters identified for the models include heating temperature, parabens concentration, and the length of the parabens' alkyl side chains (methyl, ethyl, propyl, butyl, and heptyl). The study is employing a complete block design (3 X 4 X 5 X 3) using Brain Heart Infusion as the model heating menstruum. The heating trials with *C. sakazakii* 607, a documented heat resistant strain, are conducted using a submerged coil apparatus. Survivors are enumerated by surface plating on Tryptic Soy Agar (injured + non-injured cells) and MacConkey Agar (non-injured cells). The survivor curves are then fitted using an ExpDEC1 model using with OriginPro software. While data acquisition is still underway, the results at the first inactivation treatment temperature of 58°C clearly demonstrate a significant enhancement of thermal inactivation that is concentration dependent and increases with increasing alkyl chain length. For example BHI with 32 ppm heptyl parabens underwent a ~5-log inactivation in 90 sec, 32 ppm butyl parabens underwent a ~4-log inactivation in 900 sec, and the 0 ppm control underwent a ~2.5-log inactivation in 1000 sec. Once data acquisition is complete, appropriate secondary models will be developed for prediction of thermal inactivation parameters. This work is providing insights into how inactivation models can be used to systematically describe enhanced inactivation kinetics as a result of inclusion of antimicrobial compounds or combinations of multiple process treatments.

Keywords: Parabens, Thermal inactivation, Cronobater sakazakii

[P.040]

Microbiological risk assessment of listeria monocytogenes in blue mussel (Mytilus edulis)

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Introduction

Mussels are generally regarded as high-risk products as they are likely to be contaminated with indigenous and non-indigenous pathogens and toxins. The aim of this study was to conduct a quantitative microbiological risk assessment (QMRA) of Listeris monocytogenes in the blue mussel (Mytilus edulis), following the guideline provided by Codex Alimentarius and using predictive microbiology to calculate possible growth of L. monocytogenes in mussels.

Methods

The study was based on prevalence of illness (listeriosis) and by consumer survey on blue mussel consumption in Iceland, also by collecting data on L. monocytogenes contamination in blue mussels and its environment, simulation and prediction of bacterial growth in the mussels. A quantitative risk assessment model was developed to assess the risk in healthy and susceptible population. Different assumptions were made to describe the variables of the model by probability distribution and mathematical models. Monte Carlo simulations of the model were run to estimate the number of cases in healthy and susceptible populations.

Results

Contradictory to results from abroad no L. monocytogenes were found in Blue mussels collected locally in Iceland. Consumption of mussels in Iceland is low compared to other countries. From the three risk management measures simulated, shelf life reduction and hygiene improvement with triangle distribution for the both healthy and susceptible population groups was the most effective in reducing the number of cases of illness. Bases on research data, few risk management options were provided to minimize the defined risk.

Conclusion

This work showed that the risk of acquiring listeriosis from the consumption of locally grown Blue mussels is minimal. The fresh Blue mussels were found save to consume however other hazards might pose other risks.

Keywords: Risk assessment, Listeria monocytogenes, mussels, Iceland

[P.041]

Modeling the interaction between Lactobacillus strains and Listeria monocytogenes under different temperature conditions in fish-based food matrix

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The Lactic Acid Bacteria (LAB) have an important function in food preservation, as well as their metabolites, including lactic acid, acetic acid, diacetyl, hydrogen peroxide and bacteriocins. These compounds may have antimicrobial properties reducing growth ability of pathogens and spoilage bacteria. The aim of this work was to model the interaction between LAB species and *Listeria monocytogenes* in fish extract under different temperature conditions. The paper disc method was used for observation of the inhibitory activity of different LAB species on *L. monocytogenes* based on the formation of a clear zone around the paper disc. *Lactobacillus plantarum* was selected for further study according to results obtained with the disc method. The antagonist effect of this microorganism on a cocktail of *L. monocytogenes* strains in sterile fish extract at temperatures between 4-25 ºC was quantified by using plate count method. Observations were used to fit primary growth models using as basis the Lotka-Volterra type models, which showed a good performance for both microbial populations. The use of *L. plantarum* as bioprotective culture can represent an interesting alternative for ensuring food safety of minimally processed fish products. The development of predictive microbiology models including interspecies interaction are useful tools to establish more accurate risk-based control measures.

Keywords: microbial interaction, predictive microbiology, fish extract, bioprotection

[P.042]

Comparison of a novel and existing models describing interactions between the effects of environmental conditions on the microbial growth rate

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Despite all EU efforts to tackle food poisoning and spoilage, about 5,200 outbreaks and 1.3 billion tons of food waste are reported annually, with a significant impact on the EU economy and public health. Modelling and simulation of microbial growth and inactivation as a function of processing, transportation and storage conditions is a useful tool to guarantee food safety and quality. An important share of these models are the kinetic models used to describe the growth of pathogens and spoilers as a function of time and multiple environmental conditions. The effect of multiple environmental conditions on the microbial growth rate is often described by combining their separate effects in a multiplicative way (gamma concept). This approach leads to efficient modelling methods since the combined effect of environmental conditions can be modelled by only investigating the separate effects. However, many studies have proven that interactions between these effects should be taken into account to obtain a more accurate description of the growth rate.

In this research, a novel approach for modelling the interactions between environmental conditions is compared with existing methods. As a case study, the effect of temperature and pH on the growth rate of *Escherichia coli* K12 was modelled, based on a set of bioreactor experiments performed under static environmental conditions. The considered models are the model of Augustin and Carlier (2000), the model of Le Marc et al. (2002) and a newly proposed multiplicative interaction model. This multiplicative interaction model allows the separate identification of interactions between a set of two (or more) environmental conditions. The comparison focusses on the accuracy, interpretability and extendibility of the different models.

The novel modelling approach contributes to a modelling methodology, resulting in predictive models which are (i) accurate, (ii) easily identifiable with a limited work load, (iii) extendable to additional environmental conditions and (iv) interpretable.

Keywords: kinetic models, secondary modelling, gamma concept, interactions

[P.043]

Modelling the time to fail of peach nectars formulated by hurdle technology

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The use of regression with life-data is helpful to observe whether one or more factors affect the failure time (spoilage) of a product, obtaining a model that predicts the time to fail or growth (TTF). TTF models link kinetic (lag time) and probabilistic (growth/no-growth prediction) models for selected formulation/storage conditions.

Our objective was to assess the individual and combined effects of pH, aw, and the incorporation of potassium sorbate (KS) or sodium benzoate (BNa) at selected concentrations on the microbial stability of peach nectar during storage at 25°C, in order to model and predict TTF.

Peach nectars were formulated with 40% fruit pulp and the necessary sucrose syrup and citric acid to attain a_w 0.96, 0.97, or 0.98 and pH 3.0, 3.5, or 4.0; while 0, 500, or 1000 ppm of KS or BNa were added. Nectars were stored for 60 days in glass jars at 25°C, and periodically were analyzed (standard plate as well as yeast and mould counts). The experimental design and analyses were replicated three times. Storage times that revealed microbial populations higher than 10^4 CFU/mL and signs of spoilage were registered to model TTF by survival analysis.

From the 54 combinations tested, 9 formulations (without antimicrobials) exhibited early spoilage (3 days). For the combinations formulated with 500 ppm of BNa, spoilage was detected after 30 days; much longer spoilage times were observed for 1000 ppm of KS or BNa. In general, KS was more effective in delaying spoilage when 1000 ppm were added. TTF models included individual and interaction effects of the evaluated factors and revealed good agreement among experimental and predicted data ($R^2 > 0.90$).

Survival analysis through TTF models can be used to predict spoilage time under specific factor combinations or to select factor levels for a specific shelf-life of peach nectar.

Keywords: Time to fail, Hurdle technology, Peach nectar

[P.044]

Modelling Penicillium expansum growth response to thyme essential oil at selected water activities and pH values using surface response methodology

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The antimicrobial activity of essential oils from several plants and spices has been recognized for many years. However, data on the effect of essential oils in combination with other factors on mold growth is still scarce. Additionally, there are few models to predict performance when natural preservatives are used in combination with other factors.

Our objective was to evaluate, using full factorial design, the effects of selected water activities (0.990, 0.945, or 0.900), pHs (5, 4, or 3), and thyme essential concentration (0, 25, 50, 100, up to 1000 ppm) oil on *Penicillium expansum* lag time (λ) and radial growth rate (μ) obtained by modeling mold response using Gompertz equation, and corresponding polynomial quadratic models.

Solid media (potato-dextrose agar) formulated with every studied factor combination was inoculated with 10^3 spores/ml, and incubated at 25ºC up to 30 days. Mould colony diameter was periodically measured during incubation and adjusted with Gompertz equation (using non-linear regression) to determine λ and μ. Gompertz parameters were fitted to polynomial quadratic models that include the evaluated factors, analyzing the surface response design.

Important differences in μ were observed among tested a_w, pH, and essential oil concentrations. Decreasing a_w and pH, and increasing essential oil concentration decreased μ and increased λ. At low a_w and pH, the increase in thyme oil concentration had a dramatic effect on *P. expansum* response since 25 ppm of thyme oil inhibited its growth for 30 days at 25ºC. Gompertz parameters exhibited that *P. expansum* was sensitive to the evaluated combined factors, allowing us to construct a secondary predictive growth model.

Gompertz equation adequately described mould response for tested combinations and can be utilized to evaluate their effect. Thyme oil in combination with aw and pH reduction effectively inhibited *P. expansum* growth.

Keywords: Penicillium expansum, thyme essential oil, surface response methodology

[P.045]

Combined effects of temperature, pH and water activity on predictive ability of microbial kinetic inactivation model

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It is well known that temperature is the key factor controlling the microbial survival/inactivation. However, the interactive effects of further stressing environmental conditions may influence microbial inactivation behaviour. Water activity and pH are examples of environmental factors that greatly affect bacterial thermal resistance (as water activity increases, thermal resistance decreases; as pH decreases, thermal resistance decreases).

A number of mathematical models have been proposed for describing the effects of those factors on microbial kinetics. However, the effect of possible interactions is not commonly assessed. Besides this, one should be aware about the predictive capacity of the primary inactivation model in describing microbial behaviour, when those effects are included.

The objective of this work was to include, in the inactivation model, temperature, pH and water activity effects using a black box polynomial model, aiming at accurate prediction.

Experimental data of *Listeria innocua* obtained within the temperature range of 52.5 and 65.0 °C, pH of 4.5, 6.0 and 7.5, and water activity of 0.95 and 0.99 were used for model assessment. A Gompertz-inspired model for microbial inactivation was used, with shoulder period, maximum inactivation rate and tail as parameters. The relations of such parameters with temperature, water activity and pH were purely empirical and assumed to be polynomials.

When these mathematical relationships were included in the primary kinetic model, accurate predictions of the inactivation data were attained, thus validating the predictive ability of the model expressed in terms of the stressing environmental factors studied.

Keywords: Maximum inactivation rate, polynomial functions, temperature, pH, water activity effects

[P.046]

Characterization and identification of biofilm forming bacterial isolate Shewanella sp. DDR4

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Bacteria can adhere to natural or artificial surfaces and form sessile multicellular communities known as biofilms. The natural and artificial surfaces covered by biofilms include cells and tissues of organisms, soils, sediments, pore in glaciers, thermal vent, pipelines, heat exchangers, separation membranes, and filters. In the marine environment, biofilms cover most subtidal and intertidal solid surfaces such as rocks, ships, loops, marine animals, and algae. Totally 10 bacterial isolates were obtained from three different ships anchored at the Royapuram harbour, Chennai, Tamil Nadu, India and screened for biofilm forming activity. The bacterial isolate DDR4 showed biofilm forming activity in the microtiter plate assay with a significant optical density of 0.632. Also an attempt was made to characterize the morphological, biochemical and molecular properties. Partial sequences of the 16S rRNA genes of the marine bacterial isolate was determined following the amplification of 16S rRNA genes, these sequences were aligned with the sequences of representative species of the genus *Shewanell*a sp. and phylogenetic characters of the isolate *Shewanell*a sp EF559251 with other closely related bacterial isolates were analyzed.

Keywords: Biofilm, phylogenetic tree, marine bacteria, 16S rRNA genes analysis

[P.047]

Cost, quality, and safety: Temperature optimization for leafy greens using nonlinear programming technique

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Consumption of fresh and fresh-cut leafy greens is becoming popular and has considerably increased in recent years. However, leafy green vegetables are of serious food safety concern, as these are minimally processed and are recognized as vehicles for foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes.* The shelf life of leafy greens is generally limited to one week. The objective of the study was to optimize the maximum temperature for leafy greens during supply chain taking into account the cost of refrigeration, sensory quality parameters, and microbial safety of leafy greens using nonlinear programming (NLP). Coefficient of performance (COP) for refrigeration was used to determine the cooling cost. The loss of sensory quality parameters (for fresh cut Iceberg, Romaine lettuce, and fresh-cut chicory) was expressed using Arrhenius equations. The microbial kinetics (growth and death) of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were represented by three-phase linear, log-linear and square-root models. The cost of refrigeration was the objective function to be minimized. The nonlinear constraints were the growth of pathogens, and the loss of sensory characteristics. An interactive graphical user interface, “SHELF” was developed in MATLAB software. Results suggested that pathogen growth is of more concern than sensory quality loss for a desired shelf life of 2-3 days. For sensory quality retention, Iceberg lettuce is the most susceptible to temperature abuse followed by Romaine lettuce and fresh-cut chicory. Browning is of maximum concern for Iceberg and Romaine lettuce, whereas off-odor is the biggest concern for fresh-cut chicory. *L. monocytogenes* is of greater concern than other two pathogens at refrigerated temperatures. The results would be useful for making a decision about the maximum refrigeration temperature for leafy greens in the supply chain while simultaneously taking the cost, quality, and safety into consideration.

Keywords: leafy greens, nonlinear programming, quality and safety, refrigeration temperature

[P.048]

Models for the survival of Salmonella in low-water activity, high fat food systems at 80ºC

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Low-water activity (a_w) foods are naturally dry or have been dried through processing. *Salmonella* has demonstrated an enhanced resistance to thermal inactivation and the ability to survive for long periods of time in low a_w foods. The aim of this study was to develop mathematical models that will predict the behavior of *Salmonella* in high fat, low-a_w foods held at 80°C.

We used a dried whey protein model food system homogenized with peanut oil to achieve 20% and 50% (w/w) fat concentrations. Prepared powders were equilibrated to target a_w levels between 0.11 and 0.60. A four-strain dried cocktail of *Salmonella* was inoculated, vacuum-sealed and treated at 80 °C for 11 time points up to 48h. Survival data was fitted to the Log-linear, Geeraerd-tail, Weibull, Double-weibull, Biphasic, Biphasic shoulder, and Baranyi models. The Weibull model provided the best description of the data for all a_w and fat percentage combinations ($(f_{test} < F_{table})$, R^2_{adj}, RMSE) and was selected for secondary modeling. The influence of a_w and fat content on the survival of *Salmonella* was evaluated using multiple linear regression. Secondary models were developed and validated in toasted oat cereal, animal crackers, chia powder and natural peanut butter.

Survival significantly increased with decreasing a_w. Fat content did not significantly influence survival. Prediction of survival using secondary models provided 40% of the model residuals in the acceptable zone and a correlation coefficient of R=0.49. The % bias and % discrepancy results (-6% and 27%) are consistent with those inherent in the secondary models (0.01% and 3%). The % bias results showed that the developed models had similar accuracy in predicting survival in high fat foods (-7%) and low fat foods (-6%). The models developed in this study provide information that can be used in risk mitigation strategies for *Salmonella* in low a_w foods.

Keywords: Weibull model, dry food, Salmonella, fat

[P.049]

Modeling the growth of Listeria monocytogenes and Lactobacillus plantarum in Mediterranean fish-based extracts

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Listeria monocytogenes is a foodborne pathogen which has become a prime concern for industries and governments in the last decades. Occurrence and growth of *Listeria monocytogenes* in ready-to-eat (RTE) seafood and fishery products remain a challenge both in Europe and worldwide. Predictive models can describe growth in food during storage and importantly they can be used to provide estimation of *L. monocytogenes* concentration at the time of consumption considering the influence of natural microbiota in food. This study aimed to develop microbial growth models of *L. monocytogenes* and *L. plantarum*, as potential bioprotective culture, in fish-based extracts of two Mediterranean species, such as *Sparus aurata* and *Dicentrarchus labrax*. Growth models were developed on turbidity measurements obtained with Bioscreen C, at different storage temperatures in the range 4-25ºC. The growth media corresponded to modified extracts prepared from the Mediterranean species above described by following a modified methodology proposed by Dalgaard (1995). Growth curves representing increase of microbial concentrations over time were obtained by the application of calibration curves and the Baranyi primary model was subsequently fitted to calculate kinetic parameters at the tested conditions. Model predictions provided accurate estimations for lag time, maximum growth rate and maximum density population. According to the obtained rates, *L. plantarum* showed better growth ability at the different temperatures in both fish-based extracts, signalling its potential use as bioprotective culture, even though further studies are needed to obtain definitive conclusions. Models developed herein can be applied to improve food safety and quality of minimally processed fishery products from marine aquaculture.

Keywords: Listeria monocytogenes, Lactobacillus plantarum, fish-based extracts, food safety

[P.050]

Non-linear quality modelling of Ontario hard wheat

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In recent years the wheat market is mostly driven not by agronomic values but by the end use. Modern breeding programs require screening new wheat crosses and parental candidates for quality in large numbers before selection. The evaluation of wheat breeding lines for quality involves a series of tests of varying complexity and expense. Functionality of dough is one of the most important properties for end product quality, process efficiency and breeding programs of hard winter and spring wheat. Predictive modeling of bread volume and some rheological parameters is not an easy task. There is a pressing commercial need among breeders and millers for rapid and reliable means of predicting baking quality on a small sample. The development of a prediction method can be divided into two steps. First, is to define the best quality indicators which are easy to analyse, and second, to apply proper mathematical or statistical methods to build a prediction model or regression equation. Development of prediction models should be very valuable due to objective testing of flour quality particularly when the sample amount is very limited or the number of samples to be tested is excessive. In our studies we attempted to develop predictive quality models of Eastern Canadian winter and spring wheat based on the Near-infrared spectroscopy (NIRS) combined with artificial intelligence probabilistic methods. The development of predictive quality models were performed using WEKA program. This non-linear approach allows us to predict some grain and flour quality parameters with the following precision: Bread Volume – 85%; Peak Time – 76%; Farinograph Water Absorption – 78%,; Stability – 67%; Quality Score – 86%. The details of data preparation and steps of building the Quality models will be discussed.

Keywords: wheat quality modeling, spectroscopy, artificial inteligence, data mining

[P.051]

Predicting the application frame of bio-protective cultures in yogurt

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Spoilage of dairy products, due to fungal growth, has high economic consequences for the dairy industry in terms of scrap of products, negative impact on consumer perception and shelf-life limitations. A natural way to decrease fungal spoilage problems is by using bio-protective cultures. In order to apply bio-protective cultures successfully, it is important to understand how the performance of bio-protective cultures is influenced by environmental conditions.

The aim of present study was to screen for factors affecting fungal growth and/or the performance of bio-protective cultures and to predict the growth of spoilage organisms and the performance of bio-protective cultures in various yogurt applications.

A screening for the potential influence of nine different environmental factors (protein, fat, sugar, salt, choice of starter culture, fermentation temperature, break pH, contamination level and storage temperature) on growth of two *Penicillium spp.* (*P. brevicompactum* and *P. carneum*) and performance of a bio-protective culture on growth of the molds was conducted in a yogurt system in microtiter-scale. Subsequently, growth/no growth models for four different molds and two yeasts, were developed with the variables contamination level (5-10000 spores), storage temperature (7-30˚C) and time (1-45 days). Models were developed with and without the presence of a bio-protective culture.

The screening showed that within the levels relevant in yogurt applications, the different compositions of milk bases and fermentation profiles did not affect the subsequent growth of molds or the performance of the bio-protective culture. Contrary, addition of salt, spore concentration level and storage temperature had a high effect on both the growth of the mold and the effect of the bio-protective culture. Growth/no growth models were developed and validated for six contaminants with and without influence of the bio-protective culture. The developed models can be highly useful for understanding the application frame of bio-protective cultures in yogurt.

Keywords: Bio-protective cultures, Yoghurt, Fungal growth, Growth/no growth models

[P.052]

Estimation of Aspergillus flavus growth under the influence of different formulation factors, by means of kinetic, probabilistic and survival models

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A Box-Behnken design was conducted to determine the effect of casein concentration (0, 5, or 10%), corn oil (0, 3, or 6%), a_w (0.900, 0.945, or 0.990), pH (3.5, 5.0, or 6.5), concentration of cinnamon essential oil (CEO: 0, 200, or 400 ppm), and incubation temperature (15, 25, or 35°C) on the growth of *A. flavus* during 50 days of incubation. Potato dextrose agars were prepared, adjusted to the different levels of tested factors and poured into Petri dishes, once solidified were inoculated with mold spores and incubated at studied temperatures.

Mold response was modeled using Gompertz and quadratic polynomial equations. The obtained polynomial regression model (allowed the significant ($p<0.05$) for linear, quadratic, and interaction effects for the Gompertz equation coefficients' parameters to be identified) adequately described ($R^2>0.97$) mold growth. Additionally, in order to describe growth/not-growth boundary, collected data after 50 days of incubation were classified according to the observed response as 1 (growth) or 0 (not growth), then a binary logistic regression was used to model growth interface. Mold growth probability strongly depends on casein, oil, temperature, and a_w, as well as variations of pH and CEO concentration, being lower for those systems with higher content of CEO (>180 ppm). Furthermore, survival analysis using failure time was utilized to estimate the time at which mold growth begins. The time to fail was directly related to the temperature and CEO concentration; for systems formulated with more than 200 ppm of CEO, time to fail was >30 days for low protein and fat contents.

The three tested approaches to describe *A. flavus* response, adequately predicted growth rate and lag time, or growth probability, or the time in which grow will occur. The use and selection of any of these approaches will depend on the intended application.

Keywords: Aspergillus flavus, kinetic models, probabilisticl models, survival models

[P.053]

Estimation of the temperature dependent growth parameters of Lactobacillus viridescens in culture medium with two-step modelling and optimal experimental design approaches

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In predictive microbiology, the model parameters has been estimated using the traditional two-step modeling approach (TS), in which primary models are fitted to the microbial growth data and secondary models represent the dependence of model parameters with environmental variables. The optimal experimental design approach (OED) has been used as an alternative to TS, mainly because the improvement of model identifiability and reduction of the experimental workload and costs. *Lactobacillus viridescens* is a lactic acid bacteria that is of great interest to the meat products preservation. The objective of this study was to estimate the growth parameters of *L. viridescens* in culture medium with TS and OED. The growth of *L. viridescens* was evaluated with plating count method in Man, Rugosa and Sharpe medium. For TS, the experimental data were obtained at six temperatures (4, 8, 12, 16, 20 and 30 °C); for OED, the data were obtained in four optimal non-isothermal experiments, using increasing temperatures (ITOED) (4-8-12-16 °C and 12-16-20-25 °C) and decreasing temperatures (DTOED) (16-12-8-4 °C and 25-20-16-12-8-4 °C). The optimal experiments were designed optimizing the Fisher Information Matrix. The Baranyi and Roberts, and the Square Root models were used to describe the microbial growth, in which the *b* and *Tmin* parameters (and 95% confidence intervals) were estimated from the experimental data. The parameters obtained for TS were *b* = 0.0290 (±0.0020) $h^{0.5}°C^{-1}$ and *Tmin* = -1.33 (±1.26) °C, with R^2 = 0.991; for ITOED were *b* = 0.0314 (±0.0019) $h^{0.5}°C^{-1}$ and *Tmin* = 0.12 (±0.71) °C, with R^2 = 0.995; and for DTOED were *b* = 0.0295 (±0.0019) $h^{0.5}°C^{-1}$ and *Tmin* = -1.57 (±1.05) °C, with R^2 = 0.999. The parameters obtained from the OED approach resulted in smaller confidence intervals, higher R^2, and less experimental efforts than the parameters obtained from TS approach.

Keywords: Mathematical modeling, Dynamic temperature, Microbial growth

[P.054]

Micro Hibro 2.0: A tool for assessing risks in RTE foods

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The growing demand of RTE foods by consumers has become into a great opportunity for market exploitation; however, on the other side, it is necessary the assessment of the safety and quality of this type of products. Stakeholders have interest in such assessment, although it is not a straightforward activity, as different scientific aspects and their interaction must be taken into account. Subsequently, it would be a challenge to translate this scientific knowledge into a friendly user computer tool ready to be used by stakeholders. To cover this need, the computer tool MicroHibro 2.0 has been developed to assess the risk posed by different food products. The Risk Assessment steps included in the software are Exposure Assessment, Hazard Characterization and Risk Characterization. For Exposure Assessment, different growth and inactivation predictive models are available for various microorganisms. In addition, validation of models is possible with kinetic data provided by the user. Hazard Characterization could be easily performed by selecting the appropriate dose-response model from a list of models available in the software. Finally, risk is characterized by combining previous steps, as advised by the European Commission. The tool allows for both deterministic and probabilistic approach through MonteCarlo simulation. The tool interface is based on an object-oriented approach, which enables to design and combine specific microbial phenomena occurring along the food chain such as bacterial transfer, growth, and inactivation. The software also contains the sensibility analysis feature for stochastic Risk Assessments. MicroHibro 2.0, through its flexible structure, allows food assessors and managers to input their own data, sometimes confidential and at the level of available information. MicroHibro 2.0 represents a highly valuable and friendly decision support tool for many stakeholders with interest in assuring the safety and quality of RTE foods in a global market.

Keywords: Risk assessment, MonteCarlo analysis, Sensitivity analysis, Probability model

[P.055]

Quantifying the growth variability of pathogenic and spoilage sporeforming microorganisms of interest for chilled foods using meta-analysis techniques

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Introduction

To ensure the safety and quality of non-fermented, heat-treated, chilled products with a long shelf life, it is important to control pathogenic and spoilage spore-forming microorganisms that may grow at refrigeration temperatures, among which *Bacillus cereus* and spoilage *Bacillus* spp. are of particular concern for the industry. Meta-analysis techniques can be a strong tool for evaluating the growth potential of these microorganisms under chilled conditions and for identifying control strategies.

Aim

The aim of this study was to perform a meta-analysis of growth rates of spoilage and pathogenic psychrotrophic *Bacillus* spp. found in a publicly available predictive modelling database and to use this information for predicting their growth in chilled products.

Materials and Methods

ComBase was screened for information on growth of *B. cereus* and spoilage *Bacillus* spp. in dairy matrices and laboratory media at temperatures relevant for refrigerated storage including product abuse by the consumer (0-15□C). The simple square root model of Ratkowsky (Ratkowsky et al., 1982) was fitted to the collected datasets for each species, using the Solver add-in of Microsoft® Excel 2010 after a $\log_{10}$-transformation of the data to improve the stabilisation of the variance over the entire temperature range (den Besten and Zwietering, 2012). This allowed for the estimation of the b and T_{min} parameters of the model that describes the mean growth rate as a function of temperature.

Conclusions

The quality of extracted data varied depending on the species of microorganism with best dataset obtained for *B. cereus*. The availability of data for spoilage spore-formers was relatively limited. Quantifying the variability of growth rates as a function of temperature can allow for fail-safe shelf life estimations for chilled products. The meta-analysis also helped to identify areas where limited information is available for controlling psychrotrophic spore-formers in this specific food product category.

Keywords: Bacillus, meta-analysis, growth rate, chilled foods

[P.056]

Predicting growth of weissella viridescens in culture medium under dynamic temperature conditions

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The lactic acid bacteria (LAB) are among the main spoilage microorganisms of foods, and the Weissella viridescens (formerly Lactobacillus viridescens) is well known to cause deterioration on the meat surface and in vacuum packed meat products, and under different storage conditions, even under refrigerated conditions. Therefore, this study evaluated the predictive ability of the dynamic model of Baranyi and Roberts to describe the growth of W. viridescens in culture medium (which simulates a food rich in nutrients), subjected to dynamic temperature conditions. The Baranyi and Roberts primary model was fitted to the growth curves of W. viridescens in culture medium under six different isothermal temperatures (4, 8, 12, 16, 20 and 30 °C) previously obtained in our laboratory. Four secondary models (linear, square root, exponential and Arrhenius type) were assessed to describe the influence of temperature on the growth parameters. The square root was the best model to describe the μmax parameter. For Ymax parameter, the secondary model was considered the mean values obtained experimentally in the studied temperature range. The experimental data were used to evaluate the model predictions under dynamic conditions for two different temperature profiles, NIP-1 (12-16-20-25 °C) and NIP-2 (16-12-8-4 °C). According to the statistical indexes, the model showed better predictive ability in the NIP-1, with RMSE of 0.3341, R2 of 0.9939, bias factor of 1.0046 and accuracy factor of 1.0197; the growth of W. viridescens in the NIP-2 was underestimated, indicating a fail dangerous prediction by the model. Thus, the predictive ability of the Baranyi and Roberts model was greater when the temperature was closer to the optimal growth temperature for this bacterium (30 °C). The results showed that the predictive model can be used to predict the shelf life of meat products spoiled by *W. viridescens*.

Keywords: lactic acid bacteria, Weissella viridescens, temperature, deterioration

[P.057]

Inferring the regulatory network of Salmonella typhimurium from the model organism Escherichia coli

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Systems biology is a promising field that may aid better understanding and prediction of the behaviour of food-borne pathogens. However, data are mainly available for model organisms such *E. coli* K12. To be able to exploit systems biology in predictive microbiology, we need strategies to infer data from model organisms to food-borne pathogens.

We reconstructed the regulatory network of 11 *Salmonella* strains including *S.* Typhimurium LT2 and *S.* Typhi CT18 based on the model organism *E. coli* K12. Transcription factors (TF) were linked to genes when binding sites were found in their promotor region. Protein sequences (http://www.uniprot.org/) were compared pairwise between strains and within strains and grouped in clusters where the similarity was more than 95%. We used Position Specific Scoring Matrices (PPSM) derived from known binding sites in *E. coli* and binding sites derived from experimental data of *Salmonella* available from literature. TF binding sequences were inferred by manual curation in the case of low-throughput experiments and by motif search discoveries in the case of high-throughput experiments such as ChiP-chip and ChiP-seq. The promoter regions of all the genes from the genomes of the selected organisms were scanned with the resulting PSSM and hits with a P-value lower than the corresponding optimal P-value determined using the matrix-quality tool (http://floresta.eead.csic.es/rsat/matrix-quality_form.cgi) were considered to be significant. New high-throughput data allowed the identification of potential regulatory interactions for genes in the pathogenicity islands.

In total we obtained between 2544 and 2910 potential TF-gene links depending on the strains. The networks are publically available to the community and may be useful for both microbiologists and modellers. Indeed, if cells are complex systems, it may not only be their genetic make-up that is important to predict their response to stress but also how genes are linked to each other.

Keywords: Salmonella, regulatory network, binding sites, model organism

[P.058]

Predictive modeling of the growth of Lactobacillus viridescens under non-isothermal conditions

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Food spoilage by the action of microorganisms is a major problem that can generate large economic losses to industries, making critical the application of technologies for predicting shelf life and obtain products with higher quality. The Lactic Acid Bacteria (LAB), for example *Lactobacillus viridescens*, are among the main groups of microorganisms responsible for the deterioration of refrigerated meat products, vacuum packed and under modified atmosphere. The growth of the LAB can be predicted by mathematical models, which describe the influence of various environmental factors (such as non-isothermal conditions) on microbial growth. The objective of this study was to obtain a mathematical model able to predict the growth of *L. viridescens* in non-isothermal conditions of cultivation in culture medium (MRS broth). Six isothermal growth curves (at 4, 8, 12, 16, 20 and 30 ^{o}C) were described by Baranyi and Roberts model and the dependence of maximum specific growth rate (μ_{max}) parameter on the temperature was described with the square root secondary model. The model was validated using *L. viridescens* experimental data in the temperature ranging from 5 to 11 °C and 6 to 10 °C, changing every 12 and 24 hours, respectively. The results showed that it was possible to predict safely (bias factor greater than 1) the growth of *L. viridescens* in MRS broth under non-isothermal conditions. The observed prediction deviations may have been caused by abrupt temperature changes, generating intermediate adaptation phases.

Keywords: Dynamic temperature, Non-isothermal modeling, Predictive microbiology

[P.059]

Modelling the fate of Listeria monocytogenes in beef meat stored at refrigeration temperatures under different packaging conditions

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Listeria monocytogenes is a Gram-positive anaerobic facultative food-borne pathogen which is widely distributed in nature. This bacteria is able to survive in environments with pH between 4.0 and 9.6 (optimum 6–8) and at aw levels as low as 0.90. Moreover, this pathogen can survive at temperatures below freezing. As a consequence, some types of food products have recurrently exhibited more susceptibility to *L. monocytogenes* contamination. In particular, refrigerated meats, ready-to-eat meat (RTE) foods, milk and cheeses, and seafood, have been implicated in isolated cases of listeriosis outbreaks. As *L. monocytogenes* is a microorganism of ubiquitous nature, meat products may become contaminated with this pathogen through raw materials, the processing environment and at retail markets The objective of this study was to model the fate of *L. monocytogenes* inoculated in beef at two concentrations (2.5 and 4.0 log CFU/g), packaged under aerobic, vacuum and three modified atmosphere combinations – 70%O2/20%CO2/10%N2 (MAP70/20), 50%O2/40%CO2/10%N2 (MAP50/40) and 30%O2/60%CO2/10%N2 (MAP30/60), and refrigerated at a normal temperature (4ºC) and at a mild abuse temperature (9ºC). The experimental design produced a total of 20 conditions which were done and repeated (two experiments), yielding a total of 40 survival curves. An omnibus model based on the three-parameter Weibull equation proved statistically that *L. monocytogenes* survives better in vacuum (VP) than in aerobic conditions, although without significant difference in its ability to survive in the temperature range between 4ºC and 9ºC. Furthermore, regardless of the refrigeration temperature, the presence of CO2 in package atmosphere exerted a bactericidal effect on *L. monocytogenes* cells, being approximately 1.5 log of reduction when storage time reached 10 days. Since the pathogen can survive in VP/MAP beef at refrigerated storage, there is a need of maintaining its numbers below 100 CFU/g before packaging by placing efforts on the implementation of control measures during processing.

Keywords: Modified atmosphere packaging, vacuum packaging, Weibull, survival

[P.060]

The antimicrobial effect of Rosemary and Thyme essential oils against Listeria monocytogenes in Sous Vide Cook-Chill beef during storage

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Sous vide cook-chill (SVCC) is a technology characterized by vacuum-packaging of raw or partially prepared foods before pasteurization, followed by rapid chilling and storage below 3ºC. The application of essential oils (EOs) is a strategy to control pathogens and to extend the shelf-life of products by reducing microbial levels and oxidative processes.

The aim of this study was to evaluate the efficacy of *Rosmarinus officinalis* L. (rosemary) *Thymus vulgaris* L. (thyme) EOs against *L. monocytogenes* ATCC 679, inoculated in beef processed by SVCC stored at 2ºC and 8ºC for 1, 2, 3, 7 and 14 days.

Leaves were collected (Vila Real, Portugal), dried and hydrodistilled for 3 hours in a Clevenger. Determination of minimum inhibitory concentration (MIC) assay was performed.

Beef samples (5g) of *m. longissimus thoracis et lumborum* were packaged in COMBITHERM 0.09 mm bags (HAFRI), inoculated with 0.1mL aliquots (Ca. 8-$\log_{10}$ CFU/mL), and added individually with one of each EO at MIC values. Bags were vacuum-sealed, and samples were processed at 55ºC/65 min (for 3-$\log_{10}$ reduction). *L. monocytogenes* enumeration was done according to ISO 11290-2.

A reduction of the population of *L. monocytogenes* was observed in all samples at day 3, but this reduction was more significant for OEs samples at 2ºC. At day 14, the population of *L. monocytogenes* was similar in thyme and control at 2ºC and 8ºC. Inversely, rosemary at both temperatures show a reduction of about 2-$\log_{10}$, comparatively to control.

These results support the possibility of using rosemary as natural preservative to contribute in the reduction of *L. monocytogenes* and the importance of using adequate chilling storage for maintain this pathogen at acceptable levels in view to prevent the risk for consumers.

Keywords: Essential Oils, Listeria monocytogenes, Sous vide Cook-chill, Beef

[P.061]

Multiple regression model of thermal inactivation of Clostridium perfringens spores

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OBJECTIVE. The objective was to develop a multiple regression model that includes the full uncertainty of spore thermal inactivation kinetics variables not under our control (e.g. the strain) while adjusting for the effects of experimental conditions that are either absent in practice or known and possible to control.

METHOD and RESULTS. The single regression model of logD vs. temperature showed high variability (R^2 = 43.6%, s.e. = 0.670 and severe non-constant variance (Figure 1A). Multiple regression analysis using historical data (1965-2014) from 81 strains of *C. perfringens* shows that the location of the gene that encodes the *C. perfringens* enterotoxin (*cpe*), has a large impact; the *cpe*-gene can be present on the chromosome or on a plasmid. The logD is nearly 1 higher when the *cpe*-gene is located on the chromosome compared to when the gene is absent or located on the plasmid. The model shows that it is important to adjust for this variable and for the storage time between sporulation and heating as well as for experimental conditions that do not occur in practice: enumeration medium, spore washing and heating volume. The resulting inactivation model to be used in practice is (R^2 = 76.4%, s.e. = 0.434): ***logD (minutes) = 10.55 – 0.08405 Temperature (°C) + 0.4721 $\log_{10}$ storage time (hr)*** The residual variance is greatly reduced in the multiple regression model (Figure 1B).

CONCLUSIONS AND IMPACT OF THE STUDY. The variability of a spore heat inactivation meta-model is greatly reduced by adjusting for the quality of the enumeration medium, the location of the *cpe*-gene, the period of time between obtaining spores and their heat exposure, the experimental heating volume and whether spores are washed. This model allows the food industry to calculate the probability of survival of spores at each temperature - heating time combination.

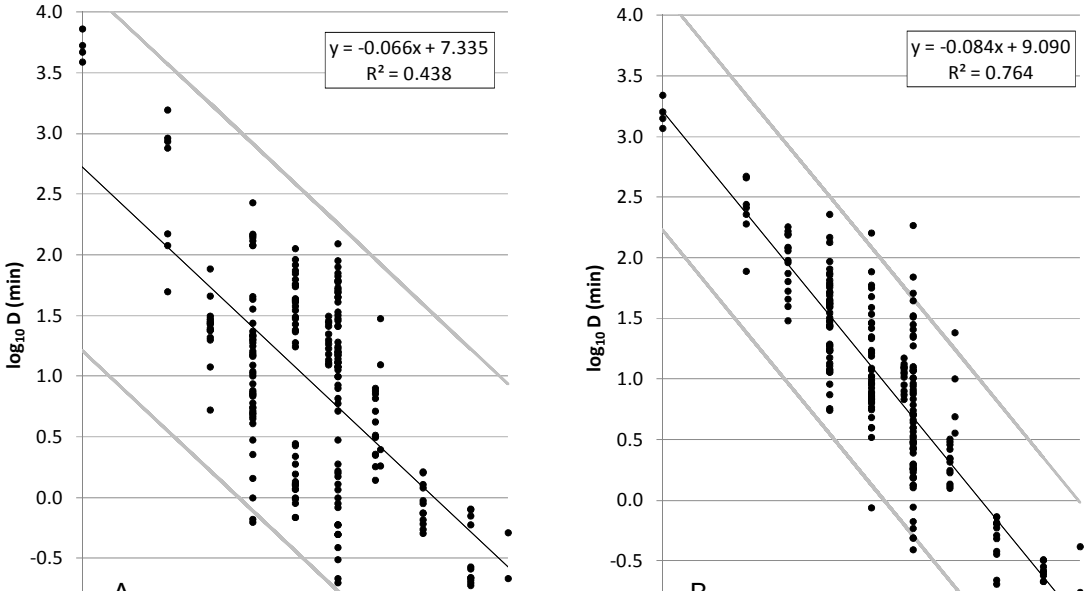

Figure 1 Regression models of $\log_{10}$ D (time to $\log_{10}$ inactivation) for temperature. A: Single regression model (s.e. = 0.670). B: Partial model for temperature from the multiple regression model (s.e. = 0.434).

[P.062]

Mathematical modeling of Lactobacillus viridescens growth in vacuum packed sliced ham under non isothermal conditions

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Lactic acid bacteria (LAB) are responsible for the spoilage of vacuum packed meat products, as ham. Temperature is the main factor affecting the microbial dynamics and its variation during the production, distribution and storage of foods is considerable. Thus, the use of mathematical models to describe the microbial behavior under variable temperatures can be very useful in predicting the food shelf life. This study evaluated the growth of *Lactobacillus viridescens* in sliced ham under non isothermal conditions, and assessed the predictive ability of the Baranyi and Roberts model using parameters obtained isothermally in culture medium (MRS). To obtain the BAL growth, the fresh ham piece was sterilized, sliced, inoculated with bacteria and stored in a temperature-controlled incubator. For the establishment of the secondary models, the primary model parameters were obtained isothermally in the culture medium at 4, 8, 12, 16, 20 and 30 ° C, in which there was no lag phase observed; the square root model was selected to describe the dependence of the μ_{max} parameter (maximum specific growth rate) with the temperature, and the y_{max} parameter (maximum population) was represented by an average because there was no significant influence of the temperature. The mathematical models were validated with *Lactobacillus viridescens* growth data in ham under five variable temperature conditions (NI-1 (4-8-12-16 °C), NI-2 (12-16-20-25 °C), NI-3 (25-20-16-12-8-4 °C), NI-4 (16-12-8-4 °C) and NI-5 (12-8-4-8-12 °C)), and its predictive ability were assessed through statistical indexes (bias factor, accuracy factor and RMSE), with good results (bias factor between 0.9450 and 1.0326; accuracy factor between 1.0382 and 1.0682, and RMSE between 0.7641 and 1.3317), especially in increasing temperature, where the prediction was safe. The validated model can be used to estimate the shelf life of a commercial ham under different temperature conditions.

Keywords: Predictive microbiology, Lactic acid bacteria, Non isothermal models, Meat products

[P.063]

Use of challenge tests to validate a new formulation of orange soft drink

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The present study aimed at validating an orange soft drink with natural colour and preservative free formula applying microbiological challenge test (MCT). The soft drink formula was submitted to physicochemical analyses such as water activity (0.96), titratable acidity (0.46%), determination of pH (3.5) and soluble solids (11.9 ºBrix). To conduct MCT, suspensions of six different microorganisms were prepared: *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, *Lactobacillus mali*, *L. plantarum*, *Gluconobacter oxydans* and *Acetobacter pasteurianus*. These strains were all isolated from spoiled soft drinks. Bottles were stored at 4ºC before inoculation. Then, triplicate beverage samples were separately inoculated with suspensions of each microorganism to reach a level of 10^4 CFU/mL, following plating on PDA (acidified Potato Dextrose Agar) (for enumeration of yeasts) and OSA (Orange Serum Agar) (for enumeration of lactic acid and acetic acid bacteria). The inoculated samples were stored at 25ºC-30ºC and were sampled after 1, 2 and 4 weeks. Negative controls were also incubated at the same conditions. For growth monitoring, decimal dilutions were made and colonies were enumerated after a 5 day incubation at 25ºC for yeasts and 30ºC/3 days for lactic acid and acetic acid bacteria. The orange soft drink formulation presented high values of the water activity, which allow microbial growth. The low pH and the presence of carbon dioxide without preservative system led to growth of yeasts and lactic acid bacteria growth within 14 days of storage, resulting in off-flavours, off-tastes and visual changes. The increase in microbial populations were of about 1 log cycle CFU/mL for lactic acid bacteria and 2 log cycle CFU/mL for yeasts after this storage period. The formulation of orange soft drink did not support the growth of acetic acid bacteria. Despite the consumer desired healthier food, the formulation of this type of products still represents a great challenge for industry.

Keywords: Soft Drink, Challenge Test, Healthier Foods, Natural Colour

[P.064]

Quantitative tools for sustainable food and energy in the food chain (Q-Safe)

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Q-Safe is an Erasmus plus project in the agro-food field. The partnership consists of seven different institutions, representing seven different countries across Europe (UK, Ireland, Belium, France, Spain, Greece, Malta (lead parner)). With rising awareness of consumer and product safety and increasing concern about food products on the market, food safety and quality worldwide faces increased pressures and challenges arising from the globalisation of food trade and intensive production systems. In this regard, predictive modelling and quantitate risk assessment is playing an ever-increasing role in food quality and safety across the globe. Recent assessments have also shown that food production has significant environmental impacts, both in terms of carbon emissions and food waste. Most of these can be assessed through Life Cycle Assessment (LCA) and related tools.

The primary aim of Q-Safe is to train early stage researchers in the area of predictive modelling, risk assessment and Life Cycle Assessment through inter-institutional cooperation. It also enhances innovative problem based learning initiatives. The simulation tools used in this partnership can be applied to various aspects in the food and bio industries, from food technologies to food management and food processing. This partnership is an excellent opportunity for early stage researchers to build scientific networks and experience, increasing collaboration, and stimulating advanced research in European academia and industry.

Towards the end of the project, 10 April to 12 April 2017, a conference will be organised in Greece to present activities of early stage researchers in the relevant research areas. For more information about Q-Safe one may refer to: http://www.um.edu.mt/healthsciences/projects/q-safe. This study reports on the advances in Q-Safe with a focus on the enhanced student experience from diverse teaching strategies adopted and intercultural exchanges.

Keywords: food chain, tools, problem based learning

[P.065]

Modelling and predicting growth of psychrotolerant pseudomonads in milk and cottage cheese

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Mathematical models were developed and evaluated for growth of psychrotolerant pseudomonads in chilled milk and cottage cheese with cultured cream dressing. The mathematical models include the effect of temperature, pH, NaCl, lactic acid and sorbic acid. A simplified cardinal parameter growth model was developed based on growth in broth. Subsequently, the reference growth rate parameter (μ_{ref} at 25 °C) was fitted to a total of 35 growth rates from cottage cheese with cultured cream dressing. Growth rate models for milk and cottage cheese were evaluated by comparison with data from literature and new experiments. Growth of psychrotolerant pseudomonads in heat-treated milk resulted in a bias factor (B_f) of 1.08 and an accuracy factor (A_f) of 1.32, whereas the calibrated model for growth rates in cottage cheese with cultured cream dressing and in raw milk resulted in B_f of 1.08 and A_f of 1.43. The acceptable simulation zone method showed the new model for cottage cheese to successfully predict growth of psychrotolerant pseudomonads at both constant and dynamic temperature storage conditions. The new models can be used together with the Food Spoilage and Safety Predictor (FSSP) software to predict growth of psychrotolerant pseudomonads and shelf-life of chilled cottage cheese and of milk at constant and dynamic storage temperatures. The developed models and the applied methodology is likely to be applicable for shelf-life assessment of other types of fermented or unripened dairy products as well as other products where psychrotolerant pseudomonads are important for spoilage.

Keywords: Cardinal parameter model, Dairy products, Model validation, Spoilage

[P.066]

Development and application of a predictive model for Alicyclobacillus acidoterrestris growth as a tool to assess risk of fruit juice spoilage

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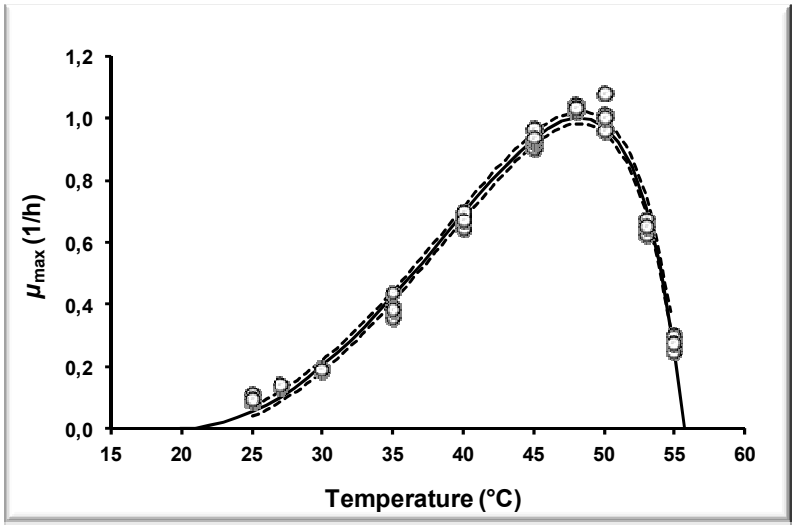
The objective of this study was to develop a predictive model for the effect of temperature and pH on *Alicyclobacillus acidoterrestris* growth and validate it in predicting spoilage of fruit juices at dynamic temperature conditions simulating distribution and storage of the product.

Aiming at modeling the behavior of *A. acidoterrestris* its growth was studied i) in K broth (2.5g/l yeast extract; 5.0g/l peptone; 1.0g/l glucose; 1.0g/l tween 80) adjusted to pH=4.5 with filtered 25% (w/v) citric acid at temperatures (*T*) ranging from 25 to 55°C and ii) in K broth of pH ranging from 3.03 to 5.53, adjusted with filtered 25% (w/v) citric acid, at the optimum growth temperature (48°C) using the turbidimetric system BioscreenC. In both cases, the growth rates were modeled using a Cardinal Model with Inflection (CMI). To adjust the model to the product, the kinetic behavior of *A. acidoterrestris* was assessed in fruit juices at 48°C and the estimated rates were used in the CMI. The model was further applied to assess the risk of fruit juice spoilage in the market of Greece based on the average daily temperature (WunderSearch® database) during a shelf life of a semester.



The estimated cardinal values of the CMI were T_{min}=19.90°C, T_{max}=55.71°C, T_{opt}=48.29°C and $\mu_{max_{opt}}$=1.001/h, respectively (Fig. 1). Simultaneously, the values for the cardinal parameters were found to be pH_{min}=2.90, pH_{max}=5.93, pH_{opt}=4.23 and $\mu_{max_{opt}}$=1.077/h, respectively (Fig. 2). Validation at dynamic temperature profiles showed that the model can accurately predict both microbial growth and time-to-spoilage.

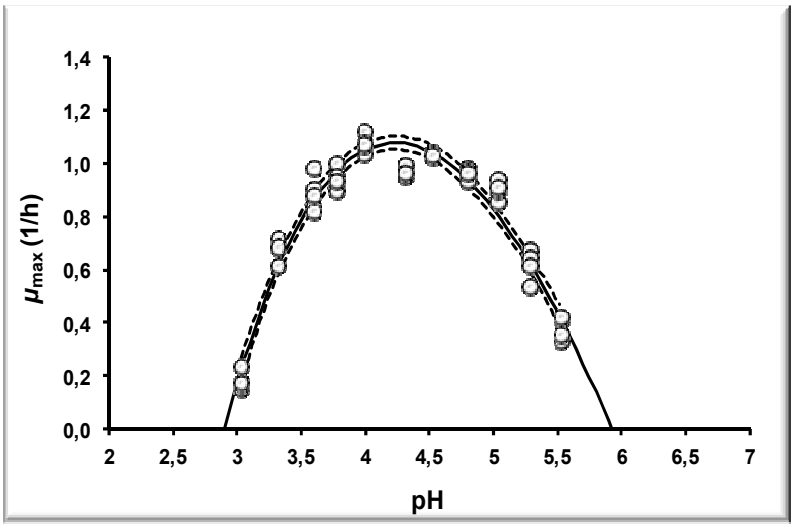


Figure 1. Effect of temperature on the maximum specific growth rate (μ_{max}) of *Alicyclobacillus acidoterrestris* ATCC 49025 in K broth, adjusted to pH=4.5 with filtered 25% (w/v) citric acid, fitted in Cardinal Model with Inflection (solid line). Points (○) represent observed values of the maximum specific growth rate. The dotted lines depict the 95% confidence limits of the effect of temperature on the maximum specific growth rate.

Figure 2. Effect of pH on the maximum specific growth rate (μ_{max}) of *Alicyclobacillus acidoterrestris* ATCC 49025 in K broth under the optimum growth temperature (T=48°C) fitted in Cardinal Model with Inflection (solid line). Points (○) represent observed values of the maximum

specific growth rate. The dotted lines depict the 95% confidence limits of the effect of pH on the maximum specific growth rate.

We demonstrated that the risk assessment model can be used by the industry as a decision-making tool for adjusting the shelf life during a semester in order to achieve an accepted risk of spoilage. This study has been co-financed by the European Regional Development Fund and Greek national funds, Project MOIKOM-09SYN-22-977.

Keywords: Alicyclobacillus acidoterrestris, growth kinetic model, time to spoilage, validation

[P.067]

Predictive microbiology models and operational readiness

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A diverse field of predictive microbiology models has emerged in the past 30 years and has advanced our understanding of microbial behavior in foods. As most of published models have for objective to provide operationally relevant information to decision makers, predictive microbiology models have now found their place within both the academic, and the food industrial communities.

Given the importance of these models to food safety, the decision makers are in need of evidence-based advices in order to assess confidence in the predictions provided by models they use. The objectives of this work were (i) to review current approaches in predictive microbiology used to build and validate models, (ii) to explore other verification and validation approaches used by other scientific fields and (iii) to propose a categorization scheme that would tend to define a model's viability for use in an operational setting.

For the first objective, a focus was done on the existing criteria (e.g. minimum number of points) to decide on the inclusion of data in modeling and on the approaches used for validation of model (dynamic condition, etc.).

For the second objective, the general scheme applied for verification and validation applied for software is presented and put in relation with tertiary predictive microbiology models.

Finally, inspired by a recent publication [Corley et al. (2014) Disease Prediction Models and Operational Readiness. PLoS ONE 9(3): e91989. doi:10.1371/journal.pone.0091989], a proposal to classify model based upon the “technology readiness level” (TRL) originally defined by NASA is made. Initial definitions of operational readiness levels for predictive microbiology models are proposed. The models would be characterized based on how the model was validated, what type of data was used to build the model, and for tertiary model how the software was verified.

Keywords: validation, software verification

[P.068]

Modified atmosphere packaging and UV-C radiation on shelf life of rainbow trout (Oncorhynchus mykiss)

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The effects of UV-C radiation, modified atmosphere packaging (MAP) and their combination on the quality of rainbow trout (*Oncorhynchus mykiss*) fillets were examined over a period of 22 days. The samples were submitted to five treatments: (T1) aerobic package; (T2) vacuum package; (T3) vacuum package + UV-C radiation; (T4) MAP (80% CO_2/20% N_2) and (T5) MAP+UV-C radiation (80% CO_2/20% N_2; 1,772 mW/cm^2). The samples were analyzed daily for microbiological (mesophilic, psychrotrophic and Enterobacteriaceae count) and chemical (pH, TBV-N, TMA-N, ammonia, lipid oxidation and biogenic amines) parameters. The bacterial groups examined presented lower growth rate and number of colonies in the stationary phase in samples submitted to MAP. T4 and T5 effectively reduced total mesophilic and psychrotrophic counts over the entire storage time. pH decrease in all treatments except T1. TBARS value increased more quickly in samples subject to T4 and T5 whereas TVB-N, TMA-N and ammonia values increased more slowly. Putrescine and cadaverine values present a similar behavior with lower production in T4 and T2 samples. In general, T4 reduced total production of ammonia, TVB-N and putrescine, whereas T5 reduced total production of TVB-N and cadaverine during entire storage time. The results of the current study suggest that MAP and MAP combined with UV-C radiation retard microbial growth and delay chemical changes enhancing the shelf life of rainbow trout fillets by at least 6 days.

Keywords: freshwater fish, UV light, MAP, predictive microbiology

[P.069]

FoodChain-Lab: Tracing software supporting foodborne disease outbreak investigations

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In case of foodborne disease outbreaks, rapid identification of the causative food product is essential, since the medical and economic damages grow with the duration of the outbreak. Recent foodborne disease outbreaks in Europe illustrated that there is a need for an expert software system capable of supporting investigations on supply chains as well as exposure assessments in crisis situations. Furthermore, the expert software system should be able to provide a comprehensive data management infrastructure assuring highest possible data quality and integrity at any point in time.

To address these needs a free, open source software called FoodChain-Lab has been developed.

Since its initial application during the EHEC outbreak in Germany in 2011 FoodChain-Lab

has been used and tested in several outbreak investigations, e.g. the Norovirus outbreak in Germany in 2012 or the Hepatitis A outbreak in Europe. On the basis of these experiences the software evolved from a data visualization and analyses tool into a comprehensive tool

box for data management, data enrichment, visualization, data analysis and interactive reasoning.

FoodChain-Lab is applicable in feed or foodborne disease outbreak investigations as well as in exposure assessment tasks related to feed or food supply chains.

FoodChain-Lab has been implemented as an extension to the modular open source data analytics platform Konstanz Information Miner (KNIME). KNIME enables visual assembly of data analysis workflows. The installation guide, the source code, example workflows and sample data are available via http://foodrisklabs.bfr.bund.de.

Keywords: tracing, foodborne disease infections, outbreaks, open-source

[P.070]

Modelling the rate of milk acidification by Lactococcus lactis ssp. cremoris influenced by protein content and type

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Predictive microbiology is mostly applied in the food safety field to model the behaviour of foodborne pathogens. However, it can also be used to describe the growth dynamics of starter cultures. The use of microfiltration (MF) enables fractionation of milk proteins, separating casein micelles from serum proteins (SP). When the process is carried out at cold temperatures β-casein migrates from casein micelles and passes through the membrane.

The aim of this study was to investigate the influence of β-casein and serum protein content on dynamics of acidification carried out by lactic acid bacteria (LAB) influenced by protein content and protein type. The following milk matrices were used to evaluate the acidification ability of LAB: skimmed milk, RMF50, RMF7 and RUF. Skim milk was microfiltered at 50^{o}C or 7^{o}C to produce, respectively, the retentate with reduced SP content (RMF50) or with reduced SP and β-casein content (RMF7). Additionally, ultrafiltration process was carried out to produce the retentate with higher casein and SP content (RUF). The gross composition of milk matrices was evaluated by using MilkoScan (FOSS). Qualitative and quantitative analysis of proteins was done by Kjeldahl and SDS-PAGE electrophoresis. The starter culture consisted of the *Lactococcus lactis ssp. cremoris* strain. The LAB were added at the level of ca. 1000cfu/ml to each milk matrice and the following parameters were measured during the acidification process (12 hours; sampling every hour): the number of LAB, lactic acid and lactose content, pH, ^{o}SH. Microbial data were subjected to primary modelling in order obtain the maximum specific growth rate.

It was concluded that higher protein content resulted in increased lactic acid production, and higher acidification rate expressed in higher values of maximum specific growth rates.

Keywords: acidification rate, growth dynamics, lactic acid bacteria, dairy products

[P.071]

The analysis of the behaviour of Listeria monocytogenes in fresh cheeses with various spices during storage

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The aim of present work was to evaluate the growth possibilities of Listeria monocytoegens in the artificially contaminated fresh cheese during storage at the following temperature: 3, 6, 9, 12 and 15oC.

Three flavor variants (mixed herbs, red pepper and garlic-pepper) of acid, homogenized commercial fresh cheeses were studied. The cheese samples were contaminated with the mixture of 3 reference strains of Listeria monocytogenes at the level of ca. 2 log cfu/g and stored at defined temperature. The frequency of sampling was determined by the applied storage temperature and the growth dynamics of Listeria monocytogenes. The primary and secondary models were elaborated on the basis of obtained microbiological data. The DMFit MS Excel add-in (www.combase.cc) was used at the stage of primary modelling by application of the growth model of Baranyi and Roberts. The square root and polynomial models were used as secondary models. The last stage of work was the mathematical validation of generated secondary models by calculation of bias (Bf) and accuracy (Af) factors.

Fresh cheeses containing different spices constituted a good matrix for the Listeria monocytogenes growth. The lowest value of the specific growth rate of L. monocytogenes in the studied fresh cheeses was reported in case of red pepper spice. The highest value of this parameter was obtained for the fresh cheese with the addition of pepper-garlic spice. The goodness-of-fit of primary models was checked by calculation of the standard error (SE) and the R-square parameter. Performed mathematical validation of the elaborated secondary models revealed the best accuracy of the polynomial model.

Keywords: Listeria monocytogenes, white cheeses, mathematical modelling

[P.072]

A case study on Bacillus amyloliquefaciens ropy bread spoilage

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Dehydrated raw materials used to produce bread are known to be highly contaminated with spore-forming bacteria. Once incremented in the bread, spores will germinate and exponential growth of amylase producing species such as *Bacillus amyloliquefaciens* may cause ropy bread spoilage in Mediterranean countries and huge economical costs. The aim of this study was to perform challenge test studies to compare growth experimental counts and *in silico* growth predictions for various bread recipes.

Based on previous work (Valerio *et al.* 2012, 2015), *B. amyloliquefaciens* ISPA-S109.3 was selected due to its resistance to the baking process and high spoilage potential. Challenge tests were performed according to standardized methods with spore inoculation (4log spore/g) in the ingredient mixture. Home bread-making machine was used to bake the 4 bread recipes, *i.e.* wheat bran bread (WBB) and white wheat bread +/- bioingredient yielding various intrinsic parameters (a_w: 0.93-0.96 and pH: 4.67-5.87). Bread incubation was performed at 20, 25 and 30°C +/- 0.01. Artificially contaminated pan bread and controls were prepared in three replicates. Distinction between ISPA-S109.3 and naturally contaminated *Bacillus* was performed using Rep PCR fingerprinting. The enumeration of ISPA-S109.3 was performed to determine growth kinetics and mathematical modeling using Sym'Previus.

Based on fitted experimental growth kinetics, growth rate in WBB at 30°C was determined (μ_{max}= 0.415+/-0.013h^{-1}) and optimal growth rate calculated (μ_{opt}=1.61+/-0.048h^{-1}) to further predict growth for the 5 tested conditions. Comparisons between experimental kinetics and growth predictions underlined that challenge test data were comprised into the 90% confidence interval of the predictions. These results highlight that major impact on growth was due to pH, a_w and temperature while the impact of food matrix determined by the μ_{opt} was sufficient, even in the case of sourdough. This study received funding from FP7-222-654-2 DREAM project and data related to behavior diversity were incremented in Sym'Previus.

Keywords: Bacillus amyloliquefaciens, Challenge test studies, in silico growth prediction

[P.073]

The new version of Sym'Previus: A friendly use of predictive microbiology

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Sym'Previus (www.symprevius.org) is a web-based decision support tool, which assists Food Business Operators (FBO's) in complying food safety and quality criteria. Sym'Previus encompasses tools to help FBOs to assess the behavior (growth and inactivation) of foodborne pathogens and spoilage microorganisms in food or during food processing. It allows both mathematical model fitting and microbial behavior prediction. Model fitting tools allow to adjust primary and secondary models to microbial inactivation and growth data. **Four tools are dedicated to the microbial behavior prediction with:**

- **the HACCP tool**. In this tool, the effect of each step of a process on bacterial growth or destruction is evaluated in order to identify the critical stages and the species that are likely to limit shelf-life.
- **the Growth interface tool**. This tool draws the growth / no growth boundaries according to the pH, water activity, temperature for the main foodborne microorganisms as well as lactic acid concentration for *Listeria monocytogenes*.
- **the growth simulation tool**. This specific tool is designed to evaluate safety and quality criteria throughout shelf-life. It allows saving time and costs associated to shelf-life determination by taken the inherent variability of food product, microorganisms and storage conditions into consideration. Furthermore, this probabilistic approach allows a tailor-made approach by using the industrial auto-control data which are often insufficiently exploited by the FBOs.
- **the thermal inactivation tool**. It's evaluates the effectiveness of a thermal profile for bacterial destruction in interaction with pH and water activity characteristics of the matrix. This model calculates the rate of reduction at the end of a treatment and the probability of a given bacterial population to survive.

Keywords: Sym'Previus, Predictive microbiology, Software

[P.074]

UV-C radiation on shelf life of hybrid tambacu (Colossoma macropomum X Piaractus mesopotamicus)

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Colossoma macropomum X Piaractus mesopotamicus hybrid is a Brazilian freshwater fish considered one of the greatest commercial importance specie in Brazil aquaculture. However, fresh fish is considered a high perishability food and conservation technologies as UV-C radiation has been studied to improve safety and extend shelf life. The effects of UV-C radiation on the quality of Colossoma macropomum X Piaractus mesopotamicus hybrid fillets were examined over a period of 6 days. The samples were submitted to three treatments: (T1) vacuum package; (T2) vacuum package + UV-C radiation (55.83 mJ/cm2); (T3) vacuum package + UV-C radiation (160.97 mJ/cm2). The samples were analyzed daily for microbiological (mesophilic, psychrotrophic and Enterobacteriaceae count) and chemical (pH, N-TVB, ammonia, lipid oxidation and biogenic amines) parameters. The bacterial groups examined presented lower growth rate and number of colonies in the stationary phase in samples submitted to UV-C radiation dose (T2 and T3). pH decreased ($p < 0.05$) only in T1 samples. UV-C treatment (T2 and T3) prevented increase of ammonia values in fish samples. The concentration of biogenic amines increased ($p < 0.05$) during the all storage time, especially in fillets submitted to UV-C radiation (T2 and T3). The results of the current study suggest that UV-C radiation enhanced the shelf-life of Colossoma macropomum X Piaractus mesopotamicus fillets at least 50% by retarding microbial growth parameters and delaying chemical changes.

Keywords: freshwater fish, UV light, Vacuum package, predictive microbiology

[P.075]

A growth model for L. monocytogenes in structured dairy products

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Introduction

The dairy industry lacks applied growth models of *Listeria monocytogenes* in cheese that cover both (a) heterogeneous food matrices possibly with both planktonic and immobilized bacteria (b) all the significant physico-chemical variables influencing microbial behavior, and (c) microbial interaction. The objective of this study was to expand an existing cardinal parameter model for the growth of *L. monocytogenes*[1] with the limiting effect of structure in a simplistic way.

Methods

In order to take into account that bacteria in cheese may exist both in a planktonic and an immobilized state, a novel growth kinetics was formulated splitting the integral population of *L. monocytogenes* into a planktonic fraction and an immobilized fraction. For including the effect of diffusion limited substrate and acidification in immobilized colonies, discrete intra-colony state variables are introduced for substrate and pH. The diffusivity of specific cheeses is estimated empirically from moisture content in cheese[2]. The predictions based on the novel type of kinetics is compared with literature data for responses of *L. monocytogenes* in cheese.

Results

Predicting literature data for responses of *L. monocytogenes* in cheese based on the novel type of kinetics improved the model accuracy compared to the existing cardinal parameter model, but apparently it only partly explains why the existing generic cardinal model have a tendency to overestimate the growth rates in cheese.

Discussion

Cheeses contaminated with *L. monocytogenes* are typically cross-contaminated after the milk is coagulated and therefore mainly the cheese surface, or the whey in aqueous cracks in the rind includes the pathogen. However, if the milk for cheesemaking is contaminated, *L. monocytogenes* could be distributed in the entire cheese, and the influence of structure could be more significant.

References

(1) Mejlholm and Dalgaard (2009). Journal of Food Protection 72, 2132-2143.

(2) Floury et al. (2010). Dairy Science &Technology 90, 477-508.

Keywords: Food structure, Cheese, Listeria monocytogenes, Growth kinetics

[P.076]

Estimation of microbial growth parameters for pseudomonas in ground pork under dynamic temperature conditions

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The majority of food processing and storage environments in the industry and the retailers are dynamic in their nature. Previous studies focusing on thermal processes have shown that the use of these realistic environments can result in the estimation of precise and accurate microbial modeling parameters. This result had a positive effect on developing modeling structures for which their validity is not violated at other non-tested experimental conditions. Another advantage of this approach is that less laborious experimental set-ups could be constructed while disadvantages may include possible non-convergence of parameters in the applied nonlinear regression and sometimes increased mathematical complexity.

The main objective of this work was to estimate microbial growth parameters simultaneously with one-step nonlinear regression and to demonstrate that the obtained parameters are of high accuracy and precision. Previously published data (Koutsoumanis et al., 2006) describing the growth of *Pseudomonas* on ground pork (pH 5.65) stored at periodically changing temperature (24 h at 0°C and 24 h

at 10°C) of 4 different profiles were used for the applied regression analysis.

The Baranyi & Roberts (1994) primary model with a square-root secondary model for growth rate were used in MATLAB. Nonlinear regression and ordinary least squares were applied. For each of the four dynamic temperature profiles, the growth rate coefficient “a” $1/(ºC^2*hr)$, the initial microbial concentration (log(cfu/mL)), and the maximum microbial concentration (log(cfu/mL)) were estimated, with root mean square error less than 10% of total span. Maximum parameter error was less than 6%. Residuals were uncorrelated and normally distributed. These results are promising for future studies in estimating growth model parameters under dynamic temperature conditions that more accurately represent commercial processes.

Keywords: microbial growth, dynamic temperature, parameter estimation, pseudomonas

[P.077]

Thermal inactivation of Alicyclobacillus acidoterrestris in different citrus juices: A meta-analysis approach

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Alicyclobacillus acidoterrestris is acidothermophilic sporeforming bacterium of great concern for fruit juice industries because of their heat/chemical resistances and spoilage potential. In order to obtain shelf-stable products, several methods have been proposed to inactivate *A. acidoterrestris* spores. Thus, the aim of this systematic review was to perform a meta-analysis as an evaluation tool of inactivation kinetics parameters of *A. acidoterrestris* in citrus juices.

A literature search was conducted and from 34 works selected, ten and two provided D-values (time at a selected temperature needed to cause 1 log cycle reduction of the target microorganism) obtained through thermal and non-thermal methods, respectively.Data collected and compiled indicated inactivation kinetics studies were performed in the range of 70-105°C. In order to allow comparison, D-values at 95°C ($D_{95°C}$) were estimated through previous determination of Z-value.

The $D_{95°C}$ values obtained were influenced by pH and soluble solid concentration varying from 1.5 to 5.7 min. The greatest coefficient of variances was observed within studies in which pH, soluble solids and acid concentration were studied. Z-values ranged from 6.1-29.1°C. The impact of variability between studies and the use of statistical distributions to represent probabilities of D-values will be further presented.

This approach will allow integration of different sources of variability such as type of juice, pH, and presence of preservatives, soluble solids content and etc. which can be very useful for industrial predictions of spoilage potential based on likely survival of *A. acidoterrestris* spores to thermal processing.

Keywords: Bigelow, Secondary model, Mixed-linear model, D-value

[P.078]

Modeling the heat inactivation of Geobacillus thermoglucosidasius in soy drink pH 6.7.

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Soy drinks added of minerals, vitamins, guns and proteins are an important nutritional source but easily prone to spore contamination. To guarantee the product stability it is required to inactivate spoilage organisms that could survive the sterilization process. From spoiled samples two *Bacillus* were isolated: *B.licheniformis* and *G. thermoglucosidasius,* both submitted to heat shock screening. *G.thermoglucosidasius* was the most heat resistant at 132ºC/2min while *B.licheniformis* was inactivated. This research aimed to model inactivation at 121, 125 and 130°C of *G.thermoglucosidasius,* isolated from spoiled soy drink, and its kinetic parameters estimation. For this purpose a spore suspension was produced and standardized at 10^8spores/mL. The capillary method was applied using sterile soy drink as substrate. Experiments were conducted in Polystat®H13L oil bath(±0.1°C). Subculture was done in Triptyc Soy Agar (Difco) at 55°C/48h. Using log of survivors x time data at each constant temperature, the survivor curves were constructed and adjusted by GInaFiT program, for kinetic parameters estimation. The z value was calculated by phantom curve. For 121 and 125ºC inactivation was linear and data were adjusted by Bigelow and Esty model. At 130ºC inactivation was no linear with an initial shoulder prior to linear inactivation (adjusted by Geeraerd model with shoulder), demonstrating that at high temperatures the protective effect of guns and proteins cause linearity deviation in the thermal inactivation. Kinetic parameters were: $D_{121ºC}$=0.75min, R^2=0.992; $D_{125ºC}$=0.66min, R^2=0.999 and time for the first log reduction at 130ºC=15.95s or 0.26min, R^2=0.991 and z=19.56ºC, and 6log reduction at 130 and 140ºC were 1.56 and 0.48min, respectively. These results suggest that if *G.thermoglucosidasius* is present in soy drinks its inactivation is extremely difficult requiring high temperatures which can alter product nutritional value. It is important to control the spore load of raw material, aiming reducing the product initial thermophilic spore level before heat treatment.

Keywords: thermal inactivation, Geobacillus thermoglucosidasius, soy drink

[P.079]

Shelf life prediction models for ready-to-eat fresh cut salads: Testing in real cold chain

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The trend towards convenience and healthy nutrition has driven sales of pre-packed leafy salads the last years. A challenge is to develop mathematical models for shelf-life prediction as tools to control and potentially extend shelf-life.

The aim was to develop and validate predictive models for shelf-life estimation of ready-to-eat (RTE) fresh cut salads in the real food supply chain.

Mixed leafy salad (lollo rosso-45%, lollo verde-45%, rocket-15%) was packed under modified atmospheres (3%O_2,10%CO_2,87%N_2) at the production plant and stored isothermally at 2-10□C. Quality assessment was based on microbial analysis, physicochemical indices and sensory scoring. Temperature dependence of quality loss rates was modelled by Arrhenius equation. Kinetic models were also based on a database developed from published data and shelf-life studies for representative leafy greens within SOPHY FP7 European Project (www.sophy-project.eu). According to field trial design, 40 samples were transported to 2 distribution centers and randomly distributed to 10 retail stores. After 2d, samples were picked and stored in consumers' refrigerators. The quality level and remaining shelf-life (SL_R) was estimated after 2-3d of domestic storage(time of consumption) based on the predictions and was compared to actual measured values of the quality indices.

Pseudomonas dominated spoilage, followed by browning and chemical changes. The effect of temperature on shelf-life was expressed by activation energy (E_a) values of 60-80kJ/mol. Effective temperature (T_{eff}) in the cold chain ranged between 4.4-9.7°C. SL_R at the time of consumption ranged between 2-8d at 4°C and was predicted within satisfactory statistical error by the kinetic models.

Using the validated models, SL_R of RTE fresh cut salad can be estimated at any point of the chill chain if the temperature history is known. Developing and validating shelf-life models can serve as an effective tool for shelf-life assessment and the development of new products in the fresh produce food sector.

Keywords: fresh-cut salads, cold chain, shelf-life, field study

[P.080]

Preliminary results on Listeria monocytogenes strain variability / sub population resistance against different commercial sanitizers

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Listeria monocytogenes is a pathogen of concern, especially in populations considered susceptible, e.g., children, pregnant, elderly and immune compromised people. Due to its psychrotrophic profile, *L. monocytogenes* can be found in a wide range of foods, like dairy and meat products. This bacterium also have the ability of biofilm formation, potentially leading to foodborne disease outbreak and economic losses. Phenomena of microorganism persistence in equipments may be due to strain variability and resistance of sub populations to cleaning and inactivation methods, like use of sanitizers. The aim of this study was to evaluate strain variability/sub population resistance of *L. monocytogenes* against sodium hypoclorite (SH) and chlorine dioxide (CD).

Three strains of *L. monocytogenes* (3724, 3735, 3739) were grown in TSB added with 0.6¨% of Yeast Extract for 24h/37°C. 1mL of suspension was placed on a 12-well plate in contact with SH and CD in 3 different concentrations (150, 300 and 500 ppm, for SH and 1, 5 and 10 ppm, for CD, respectively). For enumeration, Mueller-Hinton Agar was used and drop-plate technique was adopted (SH counts in 0, 5, 10, 15 and 20 minutes; CD counts in 0, 60, 90, 120 and 150 seconds).

Chlorine dioxide led to significantly differences in logarithm reduction only in strain 3739 (Scott-Knott test, $p < 0,05$), when 10ppm of the product was applied. Concentrations of 1 and 5 ppm were not sufficient to inhibit bacterial growth. Sodium hypoclorite was not efficient in any of tested concentrations.

These preliminary results show that commercial sanitizers may not be efficient against different strains of the same species. Besides, sanitizers concentration often used by food industries may not be efficient against pathogens. The present work will also report on influence of different types of sanitizers and their effectiveness against strains of other pathogens, such as *Salmonella.*

Keywords: sodium hypoclorite, chlorine dioxide, pathogens, persistance

[P.081]

Primary modelling of lactic acid bacteria and yeasts in a soluble soybean extract-based beverage fermented with Kefir

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The aim of this study was to model microbial growth of LAB and yeasts during fermentation of a soluble soybean extract- based beverage using the 3.0 DmFIT Excel. The preparation of the water-soluble soybean extract (soymilk) based beverage fermented with kefir included four formulations with different concentrations of soymilk and milk. LAB count was done in MRS agar supplemented with natamycin (50 mg/ml) and acidified with acetic acid 1 N until pH 5.5 ± 0.1. Yeasts were counted by surface plating on agar MEA (Malt Extract Agar) supplemented with chloramphenicol (100 mg/L) and tetracycline (100 mg/L) with pH adjusted to 6.7. From the data of LAB and yeast growth, parameters such as lag time (λ), specific growth rate (μ) and maximum population (yEnd) were obtained using the model of Baranyi and Roberts. Regarding LAB, the highest yEnd were obtained in formulations with only milk or soy extract ($p<0.05$). Formulation containing mix of milk and soy extract has led to extension of lag time in 2-3 times in comparison to formulations containing only milk or soy extract ($p<0.05$). The highest and significant growth rate was recorded in the formulation containing only soy ($p<0.05$). On the other hand, for yeasts, no significant differences on yeast lag time and yEnd were observed ($p>0.05$). The highest growth rate was observed for the formulation containing a mix of soy and milk. It was concluded that a functional beverage could be successfully produced using soymilk and kefir grains, which presented good competitive advantages in the market of products for people with lactose intolerance and probiotic food.

Keywords: Kefir, Primary modeling, soy extract, lactic acid bacteria

[P.082]

Predicting the growth of lactic acid bacteria in two mayonnaise-based deli type products using the cardinal models of the Gamma concept

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The aim of this study was to evaluate the suitability of cardinal models based on gamma concept to predict the spoilage profile of two mayonnaise-based products supplemented with chicken or smoked salmon under various chill chain conditions. The products had different pH values in the range of 4.05 to 4.7 and different concentrations of organic acids. Microbiological and molecular characterization of microbial diversity of the products by DGGE was performed in different isothermal storage conditions from 0 to 15°C.

The minimum and maximal cardinal values for pH, temperature and organic acids were obtained from the literature, while 15°C was used as the reference temperature. The models were calibrated for each product, using the μ_{ref} value of the dominant microorganism (lactic acid bacteria) in either product, at the above reference temperature. The agreement of model with data was assessed with the acceptable prediction zone criterion.

The model showed a good performance for chicken salad stored under dynamic (chill-chain) conditions during field validation. Conversely, in salmon salad, a systematic over-prediction (fail-safe) was evident, especially for products stored above 10°C. This can be partly attributed to the temperature-dependence dominance of lactic acid bacteria species. For instance, *Lactobacillus brevis* prevailed at 10^{o}C in chicken salad, while at 15^{o}C different *Lactobacillus* species were detected. As a result, the dominance of different species can alter the cardinal and reference values causing deviation of model from observed growth. These results prompted the re-calculation of some cardinal values, for the particular species of the products tested, which markedly improved the performance of the models in salmon salad.

The calibration of the cardinal parameters to particular products is required, taking into account the impact of structure-related factors, e.g. microenvironments, batch/raw material variability and microbial diversity of specific (ephemeral) spoilage organisms.

Keywords: cardinal models, Gamma concept, lactic acid bacteria, mayonnaise-based products

[P.083]

Application of predictive models to assess the influence of thyme essential oil on Salmonella Enteritidis behaviour during shelf-life of ready-to-eat turkey meats

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The recent demand for ready-to-eat (RTE) products combined with the concepts of all-natural, without artificial preservatives foods, emerge the use of plant essential oils as an alternative to increase shelf-life and safety. However, there is a lack of studies on the inactivation kinetics of these innovative antimicrobials on microorganisms inoculated in food models.

The antimicrobial effect of thyme essential oil (TEO) against Salmonella Enteritidis inoculated on sliced RTE turkey meat products was studied.

Two different formulations of RTE meats were treated with TEO at 0.1 % v/w, concentration previously proved to be sensorially acceptable by potential consumers. Products' slices were surface inoculated with Salmonella Enteritidis (~ 4-5 log CFU/g), stored at 10 and 25 °C and microbiologically analysed during shelf-life. Baranyi & Roberts and Weibull models were adjusted to data to enable the comparison of Salmonella growth and inactivation kinetics parameters on treated and untreated samples.

Salmonella behaviour on slices during storage was strongly dependent on temperature. The pathogen was able to grow on slices of all treatments during storage at 25 °C and no statistical differences were detected ($p > 0.05$) between growth parameters on treated and untreated products. At 10 °C, pathogen showed different patterns of behaviour (growth and survival), findings that could be related with the effect of the low storage temperature associated with products composition. TEO application led to higher Salmonella inactivation rates on a product exempt of chemical preservatives. Goodness-of-fit indexes (MSE and R^2) indicated that the predictive models were able to describe the growth and survival curves of S. Enteritidis on sliced meat products, treated and untreated with TEO.

The application of TEO or its incorporation on active packaging systems as a part of hurdle technology could increase RTE turkey meat products safety and shelf-life.

[P.084]

Influence of temperature and inoculum size on spores germination percent of Clostridium algidicarnis and Clostridium estertheticum

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'Blown pack' spoilage causes high economic losses for Brazilian meat industry. This research aimed to determine the influence of temperature and inoculum size on spore germination percent of *C.algidicarnis*, isolated from spoiled vacuum meat and *C.estertheticum* DSM8809, both recognized for their package blowing ability. A central composite design - 2 factors x five levels - was applied: storage temperature (-2,3,7,11, 15°C) and inoculum size(10^1,10^2,10^3,10^4 and 10^5spores/mL, previously prepared). Experiments were conducted using RCM media, in anaerobic conditions. Counts were performed daily, during 30 days, using NMP method. Spores germination percent (SGP) for each day was calculated as: SGP (%)=(count of germinated cells/initial spore inoculum)*100). In all assays, for both microorganisms, germination was observed. *C.estertheticum* germinated faster than *C.algidicarnis* at lower temperatures: at 3°C/10^2spores of inoculum, after **1** day all spores, initially inoculated, were germinated and for *C.algidicarnis,* 100% germination was reached after **4** days. In addition, at 11°C/10^2sp/mL, germination of *C.estertheticum* was slower than *C.algidicarnis,* achieving 100% after 11 days. For inoculum of 10^3spores/mL, *C.algidicarnis* reached high germination percent quickly at 15°C (52.4% after 2 days) while *C.estertheticum,* at 7°C (39% at 2 days). Increasing initial inoculum size from 10^2 to 10^4spores/mL/3°C, increased the time for total germination from 4 to 10 days for *C.algidicarnis* and from 1 to 15 days for *C.estertheticum.* These results clearly shows that tested temperatures were no enough to inhibit spores germination of both microorganisms, even if initial population is 10^1spores/ml, emphasizing the need to avoid the vacuum packed red meat clostridial spore contamination before packaging.

Keywords: psychrotrophic clostridia, vacuum pack red meat, germination percent, „blown" pack

[P.085]

Development of mathematical model for the shelf life prediction of aquacultured rainbow trout (Oncorhynchus mykiss)

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The effect of temperature on growth of spoilage bacteria in aquacultured rainbow trout was studied. The growth of *Pseudomonads,* Enterobacteriaceae and Lactic Acid Bacteria (LAB) was examined under different storage temperature (1.8-20°C). *Pseudomonads,* Enterobacteriaceae and LAB were dominant microflora of aquacultered rainbow trout. Moreover, spoilage dynamics of rainbow trout caused by dominant microflora has not yet been studied and modeled.

A new Quality Index Method (QIM) was developed for the sensory evaluation of the samples. Maximum specific growth rate (μ_{max}) was determined from viable count for each bacteria. Data from the calculated growth curves ($n=6$) were modeled by square-root equation. Independent storage trial at a constant temperature (10°C) was performed for the evaluation of the model.

The differences between calculated (based on sensory results and bacteria count) and predicted (square-root model) shelf life was found to be -9.9, -12.2 and -9.5 %, respectively when the prediction is based on the bacteria. Product validation was implemented for each bacteria regarding to literature data. The values of bias and accuracy factors were 0.87/1.16, 0.88/1.14 and 1.20/1.32, respectively. A new mathematical model suggested herein this study, is accurately validated and could be used for the prediction of raw whole un-gutted and gutted aerobically stored rainbow trout at constant and dynamic temperatures.

Keywords: Predictive microbiology, mathematical modeling, square-root model, Oncorhynchus mykiss

[P.086]

Shelf life prediction of whole and gutted rainbow trout (Oncorhynchus mykiss) under different temperatures

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A shelf life prediction model was developed for whole and gutted rainbow trout based on total mesophilic aerobic (TMA) and total psychrophilic aerobic (TPA) bacteria under different storage temperature (2, 15, 20°C). Microbiological results were compared with the sensory results of the whole and gutted samples and exhibited a good correlation for decision and prediction of the shelf life of rainbow trout.

The growth of TMA and TPA bacteria was initially modeled in accordance with logistic model and specific maximum growth rates (μmax) were recorded. To study the effect of temperature, square root type of model was used. Regarding to the results of the study, μmax (1/h) was found to be 0.047, 0.52 and 0.47 for gutted and 0.049, 0.579 and 0.57 for whole specimens. Root mean square errors (RMSE) of the model were calculated for the evaluation of the model and found to be 1.0, 0.62 and 1.18 for gutted samples and 1.01, 0.41 and 1.12 whole samples.

Product validation was performed based on the literature data. The differences between observed and predicted shelf life in accordance with square root model was indicated that the shelf life of the rainbow trout could accurately be predicted based on the TMA and TPA bacteria results of the study.

Keywords: shelf life prediction, rainbow trout, logistic model, square-root model

[P.087]

Study of deterioration of minced beef of portuguese maronesa breed stored under various atmospheres and temperatures

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The aim of this work was to study the onset of spoilage of minced meat from bovine Portuguese autochthonous Maronesa bulls stored at 2 and 8 °C, under different packaging conditions.

Fresh meat (ms. *semimembranosus* and *semitendinosus*) was obtained 24h post mortem. Meat was cut and minced and individually packed in three different conditions: air (AP), vacuum (VP) and modified atmosphere (MAP), 50% O_2 40% CO_2 and 10% N_2. After packaging, the samples were stored at 2 and 8°C and were periodically analyzed for microbiological (Mesophilic, Psychrotrophic, Lactic acid bacteria (LAB), *Enterobacteriaceae* and *Pseudomonas* spp.), chemical-physical analysis and sensory assessment of freshness (SAF) at regular intervals (days 0, 1, 3, 7, 10, 14, 21, 28) in duplicate.

The microbiota evolution was analyzed as a function of packaging and storage time using the Baranyi model. Psychrotrophic and Mesophilic, have the greatest *lag* phase, which can be associated with a delayed microbial deterioration. The grow rate of LAB, *Enterobacteriaceae* and *Pseudomonas* spp. were greater with very little or no *lag* phase. Packaging revealed to be highly important on pH and color.

The higher pH was reached in AP, and it can be associated to the higher values of high levels of TVB-N and *Pseudomonas* spp. found in AP samples.

According to SAF, it was concluded that VP was the packaging that better maintained freshness of the meat over time. After day 7 at 8ºC and day 21 at 2ºC MAP samples presented a dark color, with lower red color scores attributed by panelists.

The results showed that VP and MAP retard the microorganisms growth thereby increasing, in general, the shelf-life of the minced meat, emphasizing the fact that preservation of meat freshness was better at 2°C, mainly due to the fact that microbial growth is lower than using mild abusive temperatures (8°C).

Keywords: Meat spoilage, Baranyi model, Portuguese autochthonous Maronesa

[P.088]

Evaluation of risk based microbiological criteria for Campylobacter in broiler carcasses in Belgium using TRiMiCri

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Introduction

Campylobacteriosis is the most frequently reported foodborne zoonosis in EU. A potential solution for the reduction of consumer exposure to *Campylobacter* is establishing a microbiological criterion (MC) for *Campylobacter* on broiler meat.The aim of the present study was to evaluate risk based microbiological criteria based on the collected *Campylobacter* data in Belgium.

Methods

The freely available (http://tools.food.dtu.dk/) software TRiMiCri was applied to evaluate risk-based microbiological criteria by two approaches: the traditional one that implies microbiological limit (ML-MC) and the second one which is based on the relative risk estimate (RRL-MC). The baseline risk was estimated based on the Belgian baseline data. The input data were *Campylobacter* counts from 28 *Campylobacter* positive bathes sampled in 6 slaughterhouses.

Results

Approximately 30% of produced batches in Belgium was not complying with ML-MC (n=5, m=1000, c=0) or with MC-RRL (n=5, RRL=1). Less stringent MC decreased the percentage of non-compliance (NC) but increased the minimum relative residual risks (MRRR – the quotient of the mean risk of all batches complying with the MC and the mean risk of the whole set of batches). In both approaches number of samples *n* had only a small impact on the variation in the percentage of NC and MRRR. Results from both approaches follow the same curve when plotting percentage of NC against MRRR.

Discussion

TRiMiCri provides user friendly software to evaluate risk based microbiological criteria. The analyses performed offer a tool to make a risk based decision on the choice of the MC. An optimum criterion would combine a low MRRR with a low percentage of NC. In both approaches proportion between MRRR and percentage of NC was rather similar. The decision about the selection of the proper MC should be based on finding a balance between public health benefits and cost for poultry industry.

Keywords: Microbiological Criteria, TRiMiCri, Campylobacter, poultry meat

[P.089]

Lactic acid bacteria as a biocontrol method to reduce growth of aflatoxigenic moulds

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Aflatoxins possess significant health risks in staple foods and feeds in tropical climates. The climate change can jeopardise production of safe food by enhancing the growth of moulds and possible formation of aflatoxin in foods and feeds. Lactic acid bacteria (LAB) isolated from local fermented foods provides a potential novel approach to mitigate the mould growth and aflatoxin production, for example in maize during storage and before consumption.

In the on-going study this approach is being investigated for reducing the occurrence and bioactivity of aflatoxins in different foods and feeds. About 200 LAB strains were isolated from fermented milk and cereal products made in households in different areas of Kenya.

All LAB isolates were tested against one locally isolated S-type *Aspergillus flavus* strain. Significant inhibition showing isolates (n =19) were further tested against an *Aspergillus flavus* ATCC reference strain. One LAB isolate showed significant mould growth inhibition against both *Aspergillus* strains. Two LAB isolates showed significant mould growth inhibition against the locally isolated *Aspergillus* strain, but less against the reference strain. All LAB isolates were first identified with 16S rDNA and then subjected to analysis with recA sequencing for species- specific identification.

In further studies the identified LAB strains will be tested alone or in combination for their efficacy to inhibit *Aspergillus* growth and reduce or prevent aflatoxin formation in different food matrices and in different conditions.

This research is done as part of the FoodAfrica programme, which is a research for development programme funded mainly by the Finnish Ministry for Foreign Affairs.

Keywords: Aspergillus flavus, Lactic acid bacteria, Moulds, Inhibition

[P.090]

Modeling Salmonella inactivation in low moisture foods: Using parameter estimation to improve model performance

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Validating *Salmonella* inactivation processes for low moisture foods is a critically important food safety requirement, due to *Salmonella* persistence in these systems. Application of microbial inactivation models for this purpose is complicated by critical interactions between product water content and activity, temperature, and process humidity. Several models have been proposed; however, very few can handle or have been tested under dynamic conditions. One previously published model accounted for product surface temperature and process dew point, to predict *Salmonella* inactivation on almonds, but did not incorporate dynamic water activity. The goal of this study was to apply improved parameter estimation techniques to reduce correlation and relative standard errors of the parameters (RSEP), and to propose a more robust model for this application. Model fitting was performed using nonlinear regression, and the root mean squared error (RMSE), RSEP, variance-covariance matrix (VCM) and scaled sensitivity coefficients (SSC) were used to evaluate model performance in terms of parameter quality and robustness. Results indicated a reasonable performance of the model (RMSE = 1.6 log), with RSEP below 7.5%. However, VCM and SSC indicated correlation among the parameters. Therefore, multivariate optimization was applied to minimize the correlation, with the sum of the RSEP used as the objective function. Two of the elements on the VCM were reduced from around -0.5 to < 0.1, and the RSEP of the associated parameter also reduced from ~7.5% to < 3.5%. The remaining matrix elements did not change, which indicates an inherently larger correlation among those parameters (0.91). Post-fitting analysis of estimated parameters and optimization of reference values for inactivation models are useful to improve model performance and reliability. An attempt to reparametrize the correlated parameters, accounting for the effect of product water activity, is underway. This modification accounts for process conditions, product characteristics, and interactions with product surface temperature.

Keywords: Salmonella, Inactivation, Moisture, Parameter estimation

[P.091]

Estimation the risk of salmonella with different quantitative risk assessment models in seafood

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Risk assessment of *Salmonalla* spp. in seafood was performed in accordance with the prevalence of bacteria in various seafood namely, whole fresh chilled seabream and seabass - chilled pangasius fillets (frozen thawed), whole frozen seabream and seabass, frozen pangasius fillets and marinated anchovy, seafood salad which consist the mixture of squid, octopus, mussel, shrimp, ink fish, snailfish meat and finally smoked marinated mackerel.

Prevalence of Salmonella spp. was found higher in raw seafood products compared to processed samples. Based on the experimental data risk assessment model was developed by using online software (MicroHibro v2.0 Beta). Risk model was consisted of 4 steps (i.e transfer model during transportation, growrh model at retail level, growth model before consumption and reduction model during cooking). Monte carlo simulation was performed for the prevelance/growth of the bacteria for transportation, at retail level and during preparation. In accordance with the results of the model number of the bacteria were 0.396±0.016, 0.395±0.014 and 0.397±0.017 log cfu/g, respectively. The online tool was predicted the prevalence of *salmonella* spp. 0 % after cooking.

For the estimation and ranking of the risk a spreadsheet was used. Based on the literature data the total predicted illnesses per annum in population of interest was found to be 4940 while the risk ranking was 50. The models implemented in this study was appropriate for the risk assessment of Salmonella and could be used in production, transportation, HACCP applications and seafood safety issues.

Keywords: quantitative risk assessment model, seafood safety, monte carlo simulation, risk estimation

[P.092]

Modelling lactic acid bacteria and yeast interaction in molasses fermentation

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The bioethanol industry suffers losses due to the growth of lactic acid bacteria (LAB) during the fermentation process. Industrial fermentation is usually performed at temperatures around 30°C, which is considered optimal for the development of opportunistic microorganisms in question. The aim of this study was to evaluate the interaction between *Lactobacillus fermentum* (most frequent contaminant in fermentation process) and two different strains of yeasts at different temperatures.

The yeast strains (three type strains - *Saccharomyces cerevisiae* PE-2, CAT-1 e FT-858L; and twenty three wild strains isolated from *cachaça*), were screened for their ability to ferment sugarcane molasses at 42°C during 12 hours. Growth and viability were assessed using Neubaeur chamber and staining with methylene blue, respectively. One strain of each (type and wild strains) was selected for further sugarcane molasses fermentation at 30°C/24h. These fermentation processes were carried out in pure culture (yeast or LAB) and also in co-culture with *L. fermentum*. During the fermentation process, samples were taken at every two hours, following plating onto MEA and MRS agars by Drop Plate.

The results of screening indicated that the strain PE-2 (0,40 growth rate and viability of 99.18%) and LMQA 23 isolated by our staff (0,22 growth rate and viability of 96.46%) were more suitable for further assays. Preliminary data indicates PE-2 and *L. fermentum* co-cultured fermentation achieved higher counts (~ 10^8 CFU/mL) as compared to individual fermentation and in co-culture with the strain isolated in our laboratory.

According to preliminary results, the wild yeast isolated in our laboratory shows potential for industrial application. The next experiments will focus on the production of ethanol, organic acids, and the consumption of total sugars evaluated by HPLC, as well as the dynamics of microbial interactions in sugarcane molasses at different temperatures.

Keywords: fermentation, yeast, LAB, interaction

[P.093]

Kinetics of aflatoxin degradation during peanut roasting processing

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The aim of this study was to evaluate the kinetics of aflatoxin degradation during peanut roasting processing. Raw shelled peanuts with skin were purchased in São Paulo state, Brazil. To stimulate aflatoxin production, water activity was raised from 0.5 to 0.9 followed by incubation at 25°C for four days. The peanuts were dried until reaching initial water activity (0.5). The peanut skin was removed and 100 g portions were homogeneously separated for each roasting time and temperature analysis. The peanuts were roasted in duplicate in a vertical spouted bed roaster operating at 160, 180 and 200º C for 5, 10, 15, 20 and 25 min. Samples were milled and the three color reflectance values, CIELAB L□a□b, were measured in duplicate. Using the aflatoxin concentration quantified by high performance liquid chromatography (HPLC) after roasting, curves were drawn by regression of the log aflatoxin concentration data against the time. For 160, 180 and 200°C aflatoxin content reduction was respectively 61.6, 83.6 and 89.7%. Aflatoxin content reduction after 25 min roasting at 160ºC was adjusted using the Weibull model and the delta (δ) and *p* parameters were 120.62±127.62 and 0.59±0.37 respectively, with R^2=0.81. For 180ºC the same model was used to fit the kinetics and showed δ and *p* values of 44.58±14 and 0.7±0.27 respectively and R^2=0.92. The kinetics for 200ºC best fit was Double Weibull. This model gives two delta values: δ_1 8.23±0.27 min and δ_2 73.61±19.80. Alfa (α) and *p* parameters were 0.82±0.03 and 2.92±0.22 respectively and R^2=0.99. For the color analysis, the L* values for temperatures of 160, 180 and 200°C from 5 to 25 min of roasting ranged from 69.21 to 63.2, 60.79 to 48.41 and 57.53 to 39.29, respectively. Roasting at 160°C for five minutes was statistically similar to one of the standards analyzed.

Keywords: Aflatoxin, Kinetics, Peanut, Roasting

[P.094]

Estimation the risk associated to marketing of swine meat contaminated with Salmonella spp, employed the method of William T. Fine

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The production of swine meat is a growing market in Colombia, reaching 3.1 million of carcasses sold in 2014. However, it is estimated that the number of hogs marketed can be up to 50% higher than data reported because the illegal slaughter exposing the population to food-borne pathogens such as Salmonella. The aim of this study was to use a mathematical model to estimate the risk associated with the marketing of swine meat contaminated with nine different serotypes of Salmonella, using the method of William T. Fine. Were used survey data of Good Practice of Manufacture conducted on six-slaughterhouse pig meat and 73 outlets in the Tolima state, in the year 2010. Net production of meat sold weekly potentially contaminated and affected population was estimated. All Consequences (levels low to high), exposure to risk (low and medium) and probability of occurrence (medium) which specifies the method, was posed according to the estimate of the costs that are generated for the treatment of patients with salmonellosis were prioritized . The risk scenarios applied to the four municipalities positive for Salmonella showed a medium and low level of risk, and an exposed population of 114 thousand people representing 8.7% of the total population of the state. In the worst-case scenario, costs for hospitalization and compensation could represent more than US $ 145 billion for the biggest municipality, exceeding the available budget for a period of four years of government. It can be concluded that the method of William T. Fine is applicable for biological risk analysis focused on the costs that are generated when an outbreak occur by food illness like to Salmonella.

Keywords: Fine, Salmonella, Risk analysis, Cost analysis

[P.095]

Sequencing and identification of different Salmonella species in cocoa beans treated with gamma irradiation

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The cocoa beans (Theobroma cacao L.) are the main raw material for the production of chocolate. In the fermentation stage, is present microorganisms that help the organoleptic characteristics of cocoa, but in turn there is the presence of enterobacteria that can reach the final product. The roasting step helps to reduce the microbial load but sometimes cannot remove unwanted or pathogenic microorganisms.. The aim of this study was to evaluate the effect of different doses of gamma radiation against cocoa beans using the presence of Salmonella spp as an indicator of efectivity. Samples of cocoa beans (n=35) was treated with three different doses of cobalt 60 gamma radiation (2, 3 and 5 kGY) and without radiation as a control. All treatments were replicated three times. For the isolation of Salmonella spp was used the FDA 2007 method, maintaining a pH of 6.8 +/- 0.2. Characteristic colonies were isolated and preserved in 20 % glycerol. All isolates were reactivated on nutrient agar for subsequent DNA extraction by Boiling. The molecular identification of Salmonella was performed with primers Salm4 and Salm3, using as controls the bacteria E. coli, Pseudomonas sp. and Salmonella spp strains. Amplified isolates were sequenced and analyzed against the GenBank and RDP databank. In total, only 22 bacteria (n=124 bacteria and 372 samples with replication) were amplified with Salm3-Salm4. The alignment showed that two isolates belonged to Salmonella spp and the remaining isolates were Klebsiella sp. and Enterobacter sp. These isolates were recovered from radiation control and 2kGY, while the radiation 3 kGy and 5 kGy with an effective control in the grown of other microorganisms, mainly Salmonella. We conclude that cobalt 60 gamma irradiation is effective in reducing the bacterial load unwanted guaranteeing the microbiological quality of the product

Keywords: Radiation, PCR, Salmonella, Cocoa beans

[P.096]

Evaluation of functional probiotics' cereal flakes with fruit flavors stored at different temperatures

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Functional Probiotics foods would be promising for special uses.Cereals could design novel food products targeting young children. This investigation aimed toexamine the viability of microencapsulated Probiotics strains loaded on flakes with banana and strawberry flavors stored at 4 and 30ºC for 90 days and its acceptability after adding milk at different temperatures.

Cell suspensions with 2% active cells of either *L. paracasei441 or B. bifidumin* MRS broth were incubated at 37°C for 18h. Cells were separated by centrifugation, washed twice with saline. Suspension was mixed with an equal volume of sodium alginate (4%), added into solution of sodium chloride (0.2mol/L), calcium chloride (0.5mol/L) and magnetically stirred till alginate beads were formed. Microencapsulated cells were suspended in skim milk (10, 15, 20 and 30%), sprayed at cereal flakes with fruit flavor. Flakes were dried, packed in polyethylene pages and stored at 4 and 30ºC for 90 days. Survival rates of microencapsulated cells after adding milk at 4, 40 or 60ºC for 5 minutes as well as sensory properties were evaluated

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Cold and warm milk showed no effect on viability of *B.bifidum* and L. *paracase 441* cells after challenge for five minutes but hot milk (65˚C) decreased it. Flakes stored at 4˚C showed a good viability of both strains. Survivability of strains was decreased with prolonging storage period. Flakes moisture was stable throughout three month storage at 4˚C and 30˚C. Children well accepted cereals with Probiotics. Cereal with *B.bifidum* & banana flavor stored at 4˚C showed slightly higher organoleptic scores than at 30ºC. No difference was found between the two storage temperatures with strawberry flavor. Cereal with *L. paracasei* 441 showed close values of both flavors for the two storage temperatures. No effect was noticed for color, taste or texture.

Keywords: Probiotics, cereal flakes, viability, sensory evaluation

[P.097]

Microbiological safety of table sugar substitution with stevia in food (model) systems

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In March 2015, WHO launched a new guideline to reduce the intake of free sugars below 25 g/day. To satisfy human predisposition for sweet products, improved low-calorie sweeteners are continuously appearing in the market. In this context, stevia-based sweeteners, from a natural source, are gaining interest. While most of the studies focus on the impact of sugar substitution on human's health and food properties, little is known of its effect on food safety. Available predictive models do not consider the effect this substitution on microbial response. This work assesses the effect of table-sugar, stevia and steviol-glycosides, on *Salmonella* Typhimurium and *Listeria monocytogenes* growth dynamics in different media.

Selected sweeteners were added to minimal media, and to Tryptone Soya Broth-dextrose-free-based liquid, homogeneous and heterogeneous solid media. A full factorial design was implemented with different sweeteners´ concentrations (3, 9 and, 15%, (w/v)), temperatures (4, 8 and 20°C) and structures (liquid, homogeneous and heterogeneous solid). At regular intervals, cell concentration was determined by viable plate counting and the Baranyi and Roberts (1994) model fitted for growth parameter estimation.

Results show that the growth of *S*. Typhimurium is determined by the sweetener, its concentration, incubation temperature and media. Increasing concentrations of table-sugar in TSBdf yielded from growth reduction at 20°C, to inhibition at 8°C. This effect did not occur with stevia and steviol-glycosides. In homogeneous solid media, *S*. Typhimurium was inhibited with all the sweeteners at 8°C, while no differences occurred in heterogeneous solids. However, *L. monocytogenes* did no show significant differences between similar conditions. Results in minimal media helped to identify the different microbial use of the sweeteners.

Varying responses from the studied bacteria to changes in media formulation should be considered for future product design. Implementation of this knowledge in predictive models will allow an optimal design of food safety assurance systems.

Keywords: stevia, sweeteners, solid structures, novel food formula

[P.098]

Effect of the prehistory of cells on the inactivation efficacy of cold atmospheric plasma

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The potential of cold atmospheric plasma (CAP) for food decontamination has recently been recognized. Room-temperature gas plasmas can decontaminate foods without causing undesired changes. This innovative technology provides a worthy alternative for treating fresh produce. However, more fundamental studies of CAP inactivation and interactions with food properties are needed.

The effect of the prehistory of cells on the CAP efficacy of *Salmonella* Typhimurium and *Listeria monocytogenes* was studied. The prehistory was determined by the growth morphology, i.e., planktonic cells or surface colonies, salt concentration [0-6% (w/v) NaCl] and pH [5.5-7.4] of the medium. Cells were inactivated on a solid or in a liquid carrier, mimicking treatment of solid(like) and liquid food products. Both microorganisms were grown in petri dishes at 20°C containing Tryptic Soy Broth or Brain Heart Infusion, supplemented with 5% (w/v) gelatin for the solid systems. When cells reached the stationary phase, the cell concentration was adjusted and cells were deposited in liquid culture media or on gelatin surfaces and treated with CAP. A dielectric barrier discharge reactor generated the helium-oxygen plasma, at flow rates of respectively 4 L/min and 40 mL/min. Samples were treated up to 10 minutes at a peak voltage around 7 kV and a frequency of 15 kHz. Cell density was determined via viable plate counting on general and selective media, considering sublethal injury.

Inactivation curves were fitted with the model of Geeraerd et al. (2000). Overall, higher log reductions were obtained for cells grown planktonically under optimal conditions. More stressing growth conditions, due to cell immobilization, presence of salt or lower pH, resulted in more resistant cells during CAP treatment. This research showed that the cell prehistory influences the inactivation efficacy of CAP. This indicates that food properties affect the inactivation efficacy and need to be accounted for before CAP treatment.

Keywords: cold atmospheric plasma, cell prehistory, growth morphology, inactivation modelling

[P.099]

Estimation of dynamic metabolic fluxes in *E. coli* K12 MG1655

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Introduction: The importance of microorganisms is paramount in life sciences and industry. For food safety and legislation microbial growth has to be well controlled. In the whole cycle in which food products are produced and transported to customer, transient conditions are present (e.g., temperature shifts). Therefore accurate dynamic models for microbial growth are needed.

Materials and Methods: For an accurate modeling of microbial growth in transient conditions, microscale knowledge should also be included. In systems biology, metabolic networks are often used as tool for modeling the intracellular dynamics. Based on the principles of stoichiometric modeling a dynamic metabolic flux analysis method is applied to the dynamic estimation of the intracellular fluxes in the central carbon metabolism of *E. coli* K12 MG1655. A network consisting of 58 metabolites and 62 reactions is used, which results in 4 free fluxes to be estimated. The data required for the estimation originate from bioreactor experiments on *E. coli* K12 MG1655 with a sudden induced temperature shift from 20°C to 37°C.

Results: First, the goodness of fit on the experimental data is assessed, since this is necessary for a reliable description of the intracellular dynamics. In general, there is an accurate fit of the data, with a slight overestimation of glucose and a slight underestimation of all mass. The analysis of the metabolic pathways revealed interesting patterns in metabolic activity during different phases of growth.

Discussion: Generally speaking, knowledge of microbial growth in transient conditions, allows to accurately estimate the safety and shelf life of food products. Specifically in this case study of *E. coli* K12 MG1655, energy production is prioritized after the induced lag phase (rising glycolysis and tricarboxylic acid cycle fluxes), followed by a delayed start of the pentose phosphate pathway (production of building blocks for growth of new cells).

Keywords: dynamic metabolic flux analysis, Escherichia coli K12, induced lag phase, systems biology

[P.100]

Aerobic microbial inactivation kinetics of shrimp using fixed minimal ozone discharge: A fact or a fib during iced storage?

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Among researchers worldwide, the combination of preservation methods aimed to achieve improved effects on microbial inactivation of seafood products is an area of research receiving increasing interest. Globally also, the demand for high quality minimally processed food products are on the increase. Ozone treatment, three decade – long declared 'Generally Recognised As Safe' and approved as food contact sanitising agent has evolved up to recent times wherein assuming the likes of domestic food-processing facilities manufactured with environment-friendly status ensuring consumer safety. On the other hand, the subject of inactivation kinetics of seafood microorganisms following ozone treatment is still under debate. Furthermore, kinetic models remain quick and economical approach to predict ozone treatment parameters. Nevertheless, there is paucity of information regards microbial inactivation arising from fixed minimal ozone discharge. Is aerobic microbial inactivation kinetics of shrimp subject to a fixed minimal ozone discharge during iced storage a fact or fib? In response, the aerobic microbial inactivation of shrimp treated with ozone concentration of 100mg/h discharged minimally using wash time of 1 min during iced storage of up to 11 days was studied. The applied minimal ozone treatment was either prior to or during iced storage. The aerobic microbial inactivation trends showed significant effects during iced storage ($P<0.05$). Importantly, the line of fit that best described the aerobic microbial inactivation kinetics could only be computed using the fourth order of independent variable 'x' of storage time, which accounted for between 75 and 96% of estimated response variability. For a fact therefore, aerobic microbial inactivation kinetics of shrimp subject to a fixed minimal ozone discharge can happen even though it decreases with iced storage. (Words: 270/300).

Keywords: Minimal ozone discharge, Inactivation microbial kinetics, Storage time, Ozone efficacy

[P.101]

Effect of Undissociated Acetic and Citric acid on the growth/inactivation boundaries of Salmonella spp. in marinated chicken breast fillets stored aerobically at various isothermal conditions

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The aim of this study was the comparative investigation of the effect of pH, on the growth boundaries of *Salmonella* spp. in marinated chicken breast fillets stored aerobically as a function of undissociated acetic and citric acid molar concentration and temperature.

Chicken samples were immersed in different concentrations of lemon juice and apple cider vinegar, ranged from 0.5 to 5% (w/v) in citric and acetic acids, for 1 hr at 4^{o}C. The molar concentration range of the undissociated acids was 5.0-224.0 and 24.0-707.0 mmol/L for citric and acetic acid, respectively. After marination, samples were placed in Petri dishes and stored aerobically at 4, 8, 12, and 16^{o}C for 9 days. Changes in pH, Total Viable Counts, and *Salmonella* spp. were determined, while data were fitted to a second order logistic regression model.

Regarding inactivation boundaries, a concordance rate of 96.5 and 98.5% was predicted for the lemon juice and the apple cider vinegar, respectively. The erroneously predicted inactivation cases were 1.45 fail safe and 1.58 fail dangerous for the apple cider vinegar and 4.61% fail safe and 2.96% fail dangerous for the lemon juice. Pathogen growth was observed for concentrations below 1% (w/v) (21.0 and 64.0 mmol/L undissociated acid for citric and acetic acid) regardless of temperature, while concentrations greater than 3% (w/v) (97.0 and 358.0 mmol/L undissociated acid for citric and acetic acid) did not support growth for all cases assayed.

The developed model could be used to control *Salmonella* spp. in the tested marinated poultry products.

This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES: Reinforcement of the interdisciplinary and/or inter-institutional research and innovation

Keywords: logistic regression, Salmonella, marinated chicken fillets, organic acids

[P.102]

Modeling the effect of natamycin, resin and ecological factors on the growth, lag phase duration and OTA production by A. carbonarius using a central composite design

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The aim of this study was to model the effect of natamycin and resin on growth, lag phase duration and OTA production by *A. carbonarius*.

A Central Composite Design (CCD) and a Response Surface Methodology (RSM) were applied as a function of temperature (16.4-33.4°C), a_w (0.90-0.97), natamycin and resin for up to 1000 *ng* ml^{-1} and 2.61 % (w/v), respectively. The levels for T, a_w natamycin and resin were chosen according to a CCD (2^3 factors). The experimental design was applied with three variables, three center points, two replicates and a response surface methodology. Growth estimation was obtained by plotting the colony's radius increase against time and fitting the data with linear regression. OTA production was assessed after 5, 10 and 15 days of incubation by HPLC.

Natamycin and resin, showed a slight but not significant inhibition of fungal growth. Lag phase duration was statistically significant when treated with natamycin but not with resin. OTA production at day 15 was affected from both antifungal agents. OTA production was stimulated at lower temperatures for all a_w levels, with an exception at 20°C, 0.96 a_w and 800 ppb natamycin and 2% resin. An increase in OTA was observed with time in most cases for treatment with resin, whereas the opposite results were noticed for natamycin.

The model could be used to predict the risk of contamination by A. carbonarius and the efficacy of natamycin and resin as antifungal agents.

This work has been supported by the project 'Design and development of innovative tools for the detection of ochratoxigenic fungi in wine and table grapes – FungalPrognosis_242' co□financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) □ Research Funding Program: ARISTEIA-I.

Keywords: Aspergillus carbonarius, OTA, natamycin, resin

[P.103]

Modeling of bacterial growth in poultry meat treated with pomegranate juice marinades under dynamic temperature conditions

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A model was developed to describe the growth of total viable counts (TVC) in chicken breast fillets marinated in pomegranate juice.

Samples were immersed in pomegranate juice for 3 hours at 4^{o}C and stored aerobically at 4, 10, and 15^{o}C for 10 days. The μ_{max} of TVC was estimated at each temperature using the primary model of Baranyi. The effect of temperature on μ_{max} was further modeled using a square-root-type model. The developed models were further validated under dynamic temperature conditions using fluctuating temperature scenarios, namely (i) 16 h @ 6°C and 8 h @ 10°C (T_A) and (ii) 16 h @ 6^{o}C, 4 h @ 9^{o}C and 4 h @ 13^{o}C (T_B).

The values of μ_{max} increased from 0.088 and 0.042 (h^{-1}) at 4°C to 0.233 and 0.111 (h^{-1}) at 15°C for control and marinated samples, respectively. Graphical comparison between predicted and observed values of TVCs and the estimation of performance indices such as RMSE, accuracy (A_f) and bias factors (B_f) showed that the model predicted satisfactorily growth under dynamic temperature conditions. For marinated samples, RMSE was 0.30 and 0.42, A_f was 1.05 and 1.03, while B_f was 0.95 and 0.97 for profiles T_A and T_B, respectively. For control treatments, the values for RMSE were 0.17 and 0.19, while the A_f and B_f were 1.01 and 0.99, respectively, for both temperature profiles assayed.

The present results indicate that the model developed could be used to predict microbial growth of marinated fillets at any storage temperature.

Acknowledgment: This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES: Reinforcement of the interdisciplinary and/or inter-institutional research and innovation.

Keywords: marinated chicken, spoilage, dynamic temperature profile

[P.104]

Assessment of minced beef spoilage using Fourier Transform InfraRed (FTIR) spectroscopy, ensemble learning and Artificial Neural Networks (ANNs)

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The purpose of with this work was (a) to develop an ANN model based on spectroscopic measurements for spoilage assessment and (b) explore the efficacy of an Ensemble Learning methodology.

Fresh minced beef of two different batches was purchased from a local retailer. Portions of 70-75g were, packaged (Styrofoam trays) in air or under modified air packaging conditions (20% CO_2/80% O_2), and stored at 4 and 10°C. At appropriate intervals, four samples were analysed for total viable counts (TVC) while FTIR measurements were also collected. In total, 168 samples were analysed over 13 days.

The resulting data were pre-processed and subjected to Principal Component Analysis for dimensionality reduction. Scores were used to develop various feed-forward-NNs. Approximately 20% of the measurements were randomly retained for testing and the remaining were used for training. To avoid overfitting, early stopping criteria were employed and a small subset was used for internal validation.

Results showed that ANNs with two hidden layers performed relatively well in terms of mean squared error (MSE), however testing results were mixed. After careful selection based on MSE during calibration and internal validation, 54 ANNs were selected. Their standalone performance for the assessment of TVC in terms of MSE (log CFU/g)^2 varied from 0.25 to 1.10. Lastly, an ensemble model was created combining the former ANNs yielding an improved MSE equal to 0.22.

In conclusion, spectroscopy can be used successfully for assessment of TVC in minced beef combined with ANNs. Furthermore, the ensemble approach successfully improved model performance and avoided cases of overfitting.

This work has been supported by the project “Intelligent multi-sensor system for meat analysis-iMeatSense 550” co-financed by the European Union (European Social Fund–ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARISTEIA-I.

Keywords: Spoilage, ANN, FTIR